UNIVERSIDADE DE LISBOA

Faculdade de Medicina Veterinária

"Understanding the dynamics of African swine fever spread at the interface between wild boar and domestic swine in Sweden"

João Filipe Dias Gomes de Morais Costa

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DISSERTAÇÃO DE MESTRADO INTEGRADO EM MEDICINA VETERINÁRIA

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"Train while they sleep, study while they have fun, persist while they rest, and then live what they dream"

(Anonymous)

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"Understanding the dynamics of African swine fever spread at the interface between wild boar and domestic swine in Sweden"

Abstract

African swine fever (ASF) is a viral disease with devastating consequences that is currently spreading across the Baltic States and Poland mainly driven by wild boar cases. Countries in Europe are urged to strengthen preparedness. In Sweden, a disease spread model has been used to evaluate the risk of transmission among domestic pigs, but further investigation is needed to assess the potential spill over of ASF virus from wild boars to the domestic pig population.

This study aimed to characterize the opportunities for transmission of ASF in the interface between domestic pigs and wild boars in Sweden, providing a review of relevant information to carry out a qualitative assessment. A risk characterization using scenario trees was used, allowing similar characterizations in other countries.

Five potential transmission pathways were identified: direct contact; indirect contact; infected swill; environmental contamination/local spread; vector borne transmission. The risks identified were: geographical overlap of both populations and the domestic pig outdoor access for direct contact; human risk activities and farm biosecurity for indirect contact; swill feed ban compliance for infected swill; distance, farm biosecurity and type for environmental contamination/local spread.

This work provides a potential framework for a risk assessment of ASF in this interface, not only in Sweden but also in other countries.

Keywords: African swine fever, wild boar, domestic pig, transmission, interface, risk characterization.

"Compreendendo a dinâmica de dispersão da Peste Suína Africana no interface entre o javali e o porco doméstico na Suécia"

Resumo

A Peste Suína Africana é uma doença viral com consequências devastadoras, atualmente em dispersão nos Países Bálticos e na Polónia, particularmente devido à sua presença no javali. É, desta forma, urgente o estabelecimento de medidas preventivas por parte dos países europeus. Na Suécia, tem sido utilizado um modelo de dispersão da doença para avaliar o risco de transmissão entre porcos domésticos, sendo ainda necessários estudos que avaliem o potencial de transmissão do vírus da peste suína africana dos javalis para a população de porcos domésticos.

Este estudo pretendeu caracterizar as oportunidades de transmissão da Peste Suína Africana no interface entre javalis e porcos domésticos na Suécia, providenciando uma revisão da informação relevante para a realização de uma avaliação qualitativa. Foi utilizada uma caracterização do risco recorrendo a "árvores de evento", permitindo assim que caracterizações semelhantes sejam realizadas noutros países.

Foram identificadas cinco potenciais vias de transmissão: contacto direto, contacto indireto, restos alimentares contaminados, contaminação ambiental/dispersão local, transmissão mediada por vectores. Os riscos identificados foram: sobreposição geográfica das duas populações e o acesso ao exterior por parte dos porcos domésticos no contexto do contacto direto; atividades humanas de risco e biossegurança da exploração no contexto de contato indireto; conformidade com a proibição da alimentação com restos alimentares no contexto dos restos alimentares contaminados; distância, biossegurança da exploração e o tipo de exploração na contaminação ambiental/dispersão local;

Este trabalho fornece uma base informativa para a realização de uma análise de risco da deste interface, não só na Suécia como também em outros países.

Palavras-chave: Peste Suína Africana, javalí, porco doméstico, transmissão, interface, caracterização de risco.

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List of Abbreviations and Symbols

ASF – African swine fever
ASFV – African swine fever virus
COST - European Cooperation in Science and Technology
CSF – Classic swine fever
CVR - Central variable region
DIFT – Direct immunofluorescence test
DG SANCO - The Directorate-General for Health and Food Safety
DNA - Deoxyribonucleic acid
dNTP - Deoxynucleotides
DP – Domestic pig(s)
DTU-DADS - Technical University of Denmark - Davis Animal Disease Simulation model
EDTA - Ethylenediaminetetraacetic acid
EFSA – European Food Safety Authority
ELISA - Enzyme-Linked Immunosorbent Assay
EURL-ASF – European Union Reference Laboratory for ASF
EU – European Union
EVIRA - Finnish Food Safety Authority
FAO – Food and Agriculture Organization of the United Nations
FAO EMPRES-I – FAO Global Animal Disease Information System
FAT – Fluorescent antibody test
HAD - Haemadsorption
IFA - Indirect fluorescent antibody test
KRAV - Kontrollföreningen för Ekologisk Odling (Control Association for Alternative Cultivation)
OIE – Office International des Epizooties (World Organisation for Animal Health)
PCR – Polymerase chain reaction
PRRS - Porcine reproductive and respiratory syndrome
RF – Russian Federation
RT-PCR – Real Time PCR
UPL PCR – Universal Probe Library PCR
SVA - Statens Veterinärmedicinska Anstalt (National Veterinary Institute)
WB – Wild boar(s)

Traineeship description

The curricular traineeship was accomplished under the Erasmus Program at the National Veterinary Institute (Statens Veterinärmedicinska Anstalt [SVA]), Uppsala, Sweden, from the 3rd of October 2016 until the 31st of March 2017, in a total period of 6 months. This training was performed at the Department of Disease Control and Epidemiology with a total amount of 750 hours, supervised by Dra. Fernanda Dórea, Dr. Karl Ståhl and co-supervised by Professor Telmo Nunes (FMV- Universidade de Lisboa).

This project with the title "*Understanding the dynamics of African swine fever spread at the interface between wild boar and domestic swine in Sweden*" was a part of the research study conducted during the traineeship at the SVA.

During this period I was able to develop different research skills, such as literature review methodology, identification of information gaps, collection of information and data from different sources, collection of information through expert consultation, poster presentation, oral presentation and open discussion of the present results. A special focus was given to the methodology of each of this research steps.

I also had the opportunity to participate in the international conference "*African swine fever - recent research advances and strategies to combat the disease in Europe*" from the COST Action CA15116: Understanding and combating African swine fever in Europe (ASF-STOP) in Pulawy, Poland. In addition to having attended the lectures and discussions of the working groups 2 and 4, "ASF-Wild boar" and "ASF Epidemiology" respectively, I also had the chance to present the first results of my literature review in a scientific poster.

In parallel with the research study, practical skills of data analysis and geographical projections using the software language R were developed. It included data aggregation from different databases, structuring the data for posterior classification and analysis. However, these were only practical exercises which results were not used in this study.

During my training period, I was able to regularly visit the SVA's Department of Wildlife and Pathology, motivated by a deepest interest in wildlife and wildlife infectious diseases. During my visits to this department I could follow some of the daily clinical rounds where the responsible pathologist discuss the findings and the clinical features of the animals subjected to necropsy that day. I reserved also some time to follow and perform necropsies in wildlife carcass under the close supervision of the responsible pathologist. Beyond the practical skills, it also helped me understand the close relation between the sample collection in the pathology room and the analysis of that data by an epidemiologist. A good example of this relationship was the surveillance program of Chronic wasting disease in where samples are routinely collected from dead cervids during necropsy in order to provide data for survey in Sweden.

1. INTRODUCTION

African swine fever (ASF) is a viral disease considered as one of the most important of all swine diseases, affecting both domestic and wild pigs (Gallardo et al., 2015b; Sánchez-Vizcaíno, Mur, Gomez-Villamandos, & Carrasco, 2015a).

The ASF complexity is due to several aspects such as: wide variety of clinical forms; the existence of different genotypes; the absence of an effective vaccine or specific treatment available; existence of both wild and domestic cycles; different epidemiological behaviours and scenarios in different geographical regions (Gallardo et al., 2015b; Woźniakowski, Frączyk, Niemczuk, & Pejsak, 2016).

The spread and presence of ASF in a country or region leads to massive economic losses due to trade and transboundary restrictions, high mortality and the stamping out of animals (Gavier-Widén et al., 2015; Woźniakowski et al., 2016). Since the reintroduction of the disease in Europe in 2007, the disease has been spreading through different epidemiological scenarios until arriving to the EU (European Union) territory in 2014. Since then, the disease has been spreading mainly through wild boar (WB) populations and the eradication seems more difficult each day, with a plausible risk of becoming endemic (Bosch et al., 2016). Better understanding of the possible drivers of ASF spread is therefore crucial for all the EU members. In Sweden, a previously published foot-and-mouth disease spread model, DTU-DADS (Technical University of Denmark - Davis Animal Disease Simulation model) (Boklund, Halasa, Christiansen, & Enøe, 2013; Halasa, Boklund, Stockmarr, Enøe, & Christiansen, 2014), has been adapted to ASF and is currently being adjusted to the Swedish livestock structure by the National Veterinary Institute (Statens Veterinärmedicinska Anstalt [SVA]). The current model starts from one infected domestic pig (DP) farm and makes no assumption as to how that farm got infected. While its plausible that WB could be a source of infection, this epidemiological model does not account for ASF spread in WB population. More information is therefore needed to understand the potential role of WB in an ASF epidemic. Different authors in different epidemiological contexts have documented the spill over of ASF from WB to DP. However a contextualization of the risks in a national level is still missing

1.1 Etiology of ASF

The African swine fever virus (ASFV) is the etiologic agent of an African swine fever (ASF), a fatal and contagious disease that affects members of the *Suidae* family (Tulman et al., 2009). The ASFV is a double stranded DNA (Deoxyribonucleic acid) arbovirus, previously classified as a member of the family *Iridoviridae*, and currently classified as the sole member of genus *Asfarvirus* from the family *Asfarviridae* (International Committee on Taxonomy of Viruses & King, 2012; Tulman et al., 2009).

According to the International Committee on Taxonomy of Viruses & King (2012), this virus is morphologically composed by a "*nucleoprotein core structure, 70–100nm in diameter, surrounded by an internal lipid layer and an icosahedral capsid, 170–190 nm in diameter, and an external lipid-containing envelope*". Inside the lipid envelope is the capsid, which has an icosahedrical symmetry (International Committee on Taxonomy of Viruses & King, 2012). Its genome is composed by "*a single molecule of linear, covalently close-ended, DsDNA 170-190 Kbp in size*". The genomic differences that can happen between strains result from "*the gain or loss of members of the multigene families (MGF) located in the left and right variable regions*" (Dixon, Chapman, Netherton, & Upton, 2013).

The ASF virion has more than 50 proteins and among them enzymes and factors needed for early mRNA transcription and processing are present. Some of the virus-encoded proteins act by modulating the host response to infectious virus (International Committee on Taxonomy of Viruses & King, 2012).

The proteins responsible for the induction of antibodies after natural infectious are: the viral capsid, protein p72; two structural proteins, p30 and p54 and the polyprotein pp62 (Gallardo, Blanco, Rodriguez, Carrascosa, & Sanchez-Vizcaino, 2006; reviewed by Gallardo et al., 2015b; Pastor, Laviada, Sanchez-Vizcaino, & Escribano, 1989).

1.2 Genotyping and geographical distribution

The molecular characterization of the virus allowed to understand geographic patterns of the viral spread and, therefore, contributed in a decisive way to better understanding the epidemiology of the disease (Bastos et al., 2003; Costard et al., 2009; Costard, Mur, Lubroth, Sanchez-Vizcaino & Pfeiffer, 2013).

The current approach for molecular discrimination uses the genotyping of the B646L gene. It can use either the sequencing of the central variable region (CVR) of closely related isolates, or the PCR (Polymerase chain reaction) combination of several other gene regions in order to distinguish viral sub-groups (Bastos et al., 2003; Costard et al., 2009).

Until recently it was recognized the existence of 22 different genotypes (p72 genotypes) among the known viral isolates (Lubisi, Bastos, Dwarka, & Vosloo, 2005; Boshoff, Bastos, Gerber, & Vosloo, 2007). However, a new genotype has been recently identified in the eastern African country of Ethiopia, accounting as the 23rd p72 ASFV genotype (Achenbach et al., 2016).

All of the identified ASF genotypes circulate in Africa, with the genotype I being predominant in the eastern regions of Africa. In Europe are present at the moment two different genotypes. The genotype I is endemic in Sardinia, and, since 2007, the genotype II is also present, after the introduction and spreading from the Caucasus region (Reviewed by: Costard et al., 2009 and Gallardo et al., 2015b)

As the virus spread across Eastern Europe some molecular changes occurred. However, despite the genetic variability among the ASFV isolates circulating in Europe, Gallardo et al. (2014) considered the existence of only one ASFV variant of the genotype II circulating in Europe since 2007. The same study shows that the isolates collected from outbreaks in Lithuania and Poland in February 2014, were 100% homologous with the virus from Eastern Europe.

The isolates of the ASFV variant circulating in the Caucasus and Eastern Europe were characterized by high virulence and high mortality both in DP and WB (Blome et al., 2012; Gabriel et al., 2011). Due to this, chronic forms of ASFV and carrier states, which could contribute to long-term persistence of the disease, were ruled out. However, recently WB data collected from the north region of Estonia (Nurmoja et al., 2017), showed lower mortality and clinically healthy antibody positive WB. Subsequent experiments involving the inoculation of WB with the viral isolates collected from this region, led to the survival of one experimental animal. This animal was commingling with other three animals and no transmission was observed under the experimental conditions between the first and this latter animals. These results relaunch the discussion of viral attenuation and endemicity in the present European epidemics.

1.3 Inactivation and viral resistance

The ASFV is considered to be resistant in the environment, especially at low temperatures. It’s able to survive years at 20^0 and at 4^0C (European Food Safety Agency [EFSA], 2010; International Committee on Taxonomy of Viruses, 2012; Office International des Epizooties (OIE), 2013).

A similar relation between ASF viral survival time and environmental temperatures is seen in organic materials. In a published article assessing infectious doses (Davies et al., 2015), the

ASFV remained infectious 8.48 days at 4^0C and 3.71 days at 37^0C in faeces, and 15.33 days at 4^0C and 2.88 days at 37^0C in urine. The same authors estimate that "*the half-life of ASFV DNA was found to be 32.54 days at 4°C decreasing to 19.48 days at 37°C*".

Regarding pH, the virus was found to survive to treatments at pH4 and pH13 (International Committee on Taxonomy of Viruses & King, 2012). However according to OIE (2013) the ASFV inactivation occurs at a pH lower then 3.9 or higher then 11.5 in serum-free medium.

The ASF virion is susceptible to several chemical products such as ether, chloroform and deoxycholate. It is also inactivated by: 8/1000 sodium hydroxide (30minutes), hypochlorites – 2.3% chlorine (30 minutes), 3/1000 formalin (30 minutes), 3% ortho-phenylphenol (30 minutes) and iodine compounds (International Committee on Taxonomy of Viruses & King, 2012; OIE, 2013).

In meat products, ASFV is sensitive to heat process, but it can persist from several weeks to months if the meat is stored frozen or uncooked. It is inactivated in 3 hours at 50^0, 70 minutes at 56^0 and in 20 minutes at 60^0 (EFSA, 2010a; Mebus et al., 1997). In frozen meat products, ASFV can survive over long periods (months or years) when frozen or stored at 4°C (Dixon et al., 2005 in EFSA, 2010).

In salted dried products, the results could reflect the viral sensitivity to different food technological methods. The virus survive in Iberian and Serrano hams for 140 days, but no infectious virus was found in Parma Ham after 300 days or in cooked or canned hams when processed at 70°C (EFSA, 2010a; Farez & Morley, 1997).

1.4 Hosts

A susceptible animal is the one that becomes infected by the transmission of the agent from an infectious animal (Dohoo et al., 2009). All the susceptible hosts for ASF belong to the *Suidae* family, with the exception of the argasid ticks from the genus *Ornithodoros spp*. This argasid tick is considered to be a biological vector and reservoir for ASFV (Sanchez-Botija, 1963; Costard et al., 2013).

The members of the *Suidae* proved to be susceptible to ASF are: the domestic pigs *(Sus scrofa f: domesticus);* european wild boars *(Sus scrofa scrofa);* warthogs (*Phacochoerus africanus);* bush pigs (*Potamochoerus spp.)*; giant forest hog (*Hylochoerus meinertzhageni)* (Jori & Bastos, 2009). Only the domestic pig and the wild boar had proven to develop disease with clinical signs and mortality due to infection, while the infected African wild suids develop subclinical and asymptomatic long term persistent infections, acting as virus reservoirs (Jori & Bastos, 2009; Penrith & Vosloo, 2009; Woźniakowski et al., 2016).

The European and the African contexts represent different epidemiological scenarios, due to the presence of different hosts and vectors and due to the differences in the types of pig production. In the African context various species of wild suids act as reservoirs for the disease, condition that does not seem to occur in Europe (Bellini et al., 2016).

1.5 Pathogenesis of ASF infection

In the host the common viral entry points are the oral and nasal route. However, other routes were described, such as cutaneous, subcutaneous, tick bites and scarification (Reviewed by: Gallardo et al., 2015b). In transmission experiments with WB, both oral and intramuscular infection route lead to 100% lethality among this species (Gabriel et al., 2011; Guinat et al., 2014; Pietschmann et al., 2015).

The tonsils, the dorsal pharyngeal mucosa to the mandibular or retropharyngeal lymph nodes are the sites initial targets for viral infection. From there it spreads through blood stream and in 8 hours after the infection occurs the first vireamia. Between 15 to 24 hours afterwards, a second viraemia leads to the viral spreading from the primary sites, and the virus reach almost every tissue of the body. Around 30 hours after the virus enters the body it can be found in almost every organ. The most common are: spleen, kidneys, bone marrow, liver, lungs and the endothelium of the organs associated with the mononuclear phagocytic system (Reviewed by: Blome et al., 2013).

The ASFV infects and replicates on the cytoplasm of the mononuclear phagocyte system cells, predominantly, but not exclusively, in the macrophages and the monocytes lineage. This leads to the release of pro-inflammatory cytokines and to coagulopathy disorders. No differences in cell tropism or organ distribution had been showed comparing moderate and highly virulent strains. However, more severe tissue destruction has been associated with increased virulence (Oura, Powell, & Parkhouse, 1998; Reviewed by: Blome et al., 2013)

According to the stages of infection described by Dohoo, Martin & Stryhn (2009), the latent period is the period of time when the infectious agent is present but the individual is not capable of transmitting the infection. For pig-to-pig transmission, the latent period of ASF was experimentally estimated to be 4 days using an isolate (Georgia 2007 strain) from the current genotype circulating in the present European epidemics (Guinat et al., 2016a) and 3 to 6 days with a less virulent isolate (Malta 1978) (de Carvalho Ferreira et al., 2013a). For WB-to-WB transmission, the duration of the latent period was estimated to be 4 days using a highly virulent strain (Armenia 2008) (Pietschmann et al., 2015).

The infectious period is defined as the period of time where the host is capable of transmitting the agent (Dohoo et al., 2009). The ASF's experimentally estimated infectious period for pig-

to-pig transmission has a minimum duration of 3 to 6 days by direct and indirect contact and a maximum duration of 3 to 14 days. These results were obtained using a highly virulent and current strain (Georgia 2007 strain) (Guinat et al., 2016a). For WB-to-WB transmission, with another isolate of the current European genotype II (Armenia 2008 strain), Pietschmann et al. (2015) pointed an infectious period of 4 to 10 days.
Different results have been reported for the incubation period, which is defined by Dohoo et al. (2009), as the length of time between the infection and the onset of the clinical signs. While Gallardo et al. (2015b) published that the incubation period last from 4 to 19 days, Blome et al. (2012) reported a duration of 2 to 7 days, seldom up to 14 days, during an experiment with a high virulent strain.

1.6 Clinical forms

Animals of all ages are equally susceptible to ASF, unlike classic swine fever (CSF) that affects mainly young animals. The infection with different viral strains could be related with the different clinical course of the disease. Highly virulent strains are related with peracute, acute forms and high mortality, while moderately virulent strains are related with acute and sub-acute forms (Reviewed by: Gallardo et al., 2015b).

1.6.1 Peracute forms

The peracute clinical forms are caused by highly virulent ASFV strains. These clinical forms are characterized by: high fever (body temperature can raise to 41-42^{0}C), anorexia, inactivity, hyperpnoea and cutaneous hyperaemia. The death of the animals comes suddenly in 1 to 4 days after the onset of the clinical signs. No lesions are evident in the organs (Reviewed by: Sánchez-Vizcaíno et al., 2015a).

1.6.2 Acute Forms

These forms are caused by highly or moderately viral strains of the virus. They are characterized by: fever (body temperature of 40-42^{0}C), anorexia, apathy, mucoid nasal discharge, epistaxis, incoordination, erythema and cyanosis of the skin (Reviewed by: Gallardo et al., 2015b and Sánchez-Vizcaíno et al., 2015a)
The erythema is usually located in the skin of the ears, tails, extremities, chest, abdomen and perianal region (Reviewed by: Gallardo et al., 2015b and Sánchez-Vizcaíno et al., 2015a). The cyanosis commonly onsets 1 to 2 days before death and is located predominately on the ears, abdomen and perianal region. Small foci of cutaneous necrosis and/or subcutaneous hematomas may occur (Reviewed by: Gallardo et al., 2015b and Sánchez-Vizcaíno et al., 2015a). Signs of functional failure of the internal organs can also be present, mainly in the

digestive system with vomiting and haemorrhagic diarrhoea (Reviewed by: Gallardo et al., 2015b and Sánchez-Vizcaíno et al., 2015a). The reproductive signs are usually in form of abortion in pregnant sows (EFSA, 2009).

Between 90 to 100% of the animals die in shock, usually 1 week after the onset of the fever with foam being found sometimes around the mouth and nose (Sánchez-Vizcaíno et al., 2015a; Villeda, Williams, Wilkinson, & Vinuela, 1993).

At the necropsy, the acute form can present itself by: hyperaemic splenomegaly; haemorrhages in organs (usually visceral lymph nodes and kidney); and haematic free fluids in the body cavities (Reviewed by: Gallardo et al., 2015b and Sánchez-Vizcaíno et al., 2015a).

1.6.3 Sub acute forms

The sub acute forms are usually related with moderately virulent strains. Fever is usually persistent or fluctuating, lasting up to 20 days. In general, the affected animals show similar clinical signs as seen in the acute form, however they are usually less severe. Sometimes abortion can be the first clinical sign (Reviewed by: Sánchez-Vizcaíno et al., 2015a)

The mortality rate is between 30 and 70% after 20 days post-infection. At the necropsy the lesions are usually milder than those described in the acute form (Reviewed by: Gallardo et al., 2015b and Sánchez-Vizcaíno et al., 2015a).

1.6.4 Chronic forms

The chronic forms are caused by an infection with a low virulent strain. It has no specific clinical signs, but can lead to necrotic lesions on the skin and to arthritis (Sánchez-Botija, 1982 in Sánchez-Vizcaíno et al., 2015a), delayed growth, emaciation, lameness, respiratory signs, abortion and low mortality (Manso Ribero, Nunes Petisca, Lopes Frazão, & Sobral, 1963; Sánchez-Botija, 1982; Arias et al., 1986).

At the necropsy, it's usual the absence of vascular lesion and the presence of injuries in which the bacteria are involved, such "*as fibrinous pleuritis and/or pericarditis, pleural adhesions, necrotic pneumonia, fibrinous arthritis/periarthritis and necrotic skin lesions, as well as necrotic areas on the tonsils and the tongue*" (Moulton and Coggins, 1968; Arias et al., 1986).

1.7 Specific treatment and Vaccine

Until now, neither commercial vaccine nor specific treatment is available and therefore, avoiding the introduction of the disease in free areas is still a keystone of prevention (Bellini et al., 2016; Sánchez-Vizcaíno et al., 2012).

For several years different strategies and approaches were employed, without the production of an effective vaccine (Gallardo et al., 2015b; Sánchez-Vizcaíno et al., 2012). In the 60's the use of live attenuated vaccines led to chronic clinical forms on the Iberian Peninsula due to the infection with low virulence ASFV (Manso Ribeiro et al, 1963; Sánchez-Vizcaíno et al., 2012).

Until now, all the different attempts to produce the vaccine only led to the delay of the onset of the clinical signs and consequently the delay of the death of the animals. Only partial protection against homologous virus was achieved. None of the published studies could reach the adequate and required properties of an effective vaccine (Reviewed by: Sánchez-Vizcaíno et al., 2012).

In the present European epidemics, especially in the EU affected members, where the disease spread had been driven mainly through WB populations new challenges arise. Biosecurity measures to avoid the introduction and limit the disease expansion are the corner stones of the ASF prevention and management. However, other approaches are being considered, such as WB reproduction control (Penrith & Vosloo, 2009; Woźniakowski et al., 2016).

1.8 Diagnosis

The clinical diagnosis of ASF isn't easy due to a wide spectrum of clinical signs in the different hosts, which can also mimic other diseases. The possible differential diagnoses with ASF are: CSF; porcine reproductive and respiratory syndrome (PRRS); swine erysipelas; septaecemic salmonellosis; porcine dermatitis; nephropathic syndrome; and other septaecemic conditions like poisoning (Gallardo et al., 2015b; Woźniakowski et al., 2016).

The exclusive clinical diagnosis is often a hard task, especially if a small number of animals are affected (Sánchez-Vizcaíno et al., 2015a). However, the investigations of the clinical signs and high fatality rate in pigs have a key role in the early diagnosis of ASF in passive surveillance programs (Directorate General for Health and Food Safety [DG SANCO], 2013).

Due to the clinical diagnosis complexity, the laboratory diagnosis is a key tool in the ASF prevention and control. A sensitive laboratory diagnosis together with an appropriate interpretation provides precious information for designing effective preventive and control programs (Gallardo et al., 2015b). The laboratory diagnosis addresses two different, but complementary, perspectives: the identification of the agent and the antibody detection through serologic tests (OIE , 2012).

It's essential to perform both approaches in parallel, in order to provide a complete view of the epidemiological context in study. When ASF occurs with highly virulent strains and with peracute clinical forms, it's common that the animals' death occur before the onset of antibodies production. Despite this fact, serological tests of hunted and/or found dead animals are essential, since it can help to have a complete picture of the timeline of the outbreaks (Gallardo et al., 2015b; Guinat et al., 2016a; Woźniakowski et al., 2016).

1.8.1 Viral identification

In laboratory diagnosis of ASFV, the virus is isolated in porcine macrophage cultures obtained from blood, bone marrow and lungs. Infected macrophages show cytopathic and often haemadsorption effect that are used also to quantify the virus through "in vitro" viral titration (Malmquist & Hay, 1960).

Currently, the available validated diagnostic tests for virus detection are (Gallardo et al., 2015b): Virus isolation/Haemadsorption (HAD); FAT test; Antigen ELISA (Enzyme-Linked Immunosorbent Assay) K2; PCR conventional-Aguero; Real time PCR-King; UPL PCR [Universal Probe Library PCR]; PCR Multiplex; PCR Tignon; PCR tetracore; Tetracore/ARS.

Isolation is recommended when there is a suspicious case of ASF in a country considered free. If tissues are unsuitable for virus isolation and antigen detection, PCR is recommended.

The samples from the suspected animals should contemplate: blood in anticoagulant (Ethylenediaminetetraacetic acid [EDTA]); spleen; lymph nodes; tonsil; kidney (OIE, 2013)

PCR tests are the first choice for early detection of ASF viral genome, according to Gallardo et al. (2015a), since they are an excellent, highly sensitive and rapid technique for the ASFV detection. They are useful under a wide range of circumstances, especially if the tissues are not suited for viral isolation and/or for antigen detection (OIE, 2012).

1.8.1.1 Haemadsorption Test (HAD)

According to the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (2012), the virus isolation through haemadsorption test is based on the principle of adhesion of pig erythrocytes to the surface of the pig monocyte or macrophage cells infected with ASFV.

The majority of ASF strains produce "*the haemadsorption reaction (HAD) due to adsorption of pig red blood cells on ASFV infected leukocytes*". While a positive result in this test confirms the ASF diagnosis, a small number of non-haemadsorbing virus have been isolated, some of which produce a typical acute clinical form of ASF. This reaction is of particular importance since none of the other swine virus is capable of producing a haemadsorption reaction in leukocyte cells (European Union Reference Laboratory for ASF [EURL-ASF], 2013a).

Given to its long and laborious procedure, this method isn't recommend as a first choice for primary diagnosis. This test is recommended as a reference for confirming primary positive results through other methods (ELISA, PCR, Direct Immunofluorescence tests). False positive reactions could occur due to poorly conserved samples (EURL-ASF, 2013a).

1.8.1.2 Antigen detection by fluorescent antibody test (FAT)

The FAT method is based on the UV light microscopic detection of viral antigens on impression smear of organ samples from suspicious animals using positive and negative controls. The intracellular antigen (inclusion bodies) are detected using anti-ASF specific sera labelled with fluorescein isothiocyanate (Reviewed by: Oura et al., 2013).

A positive test together with relevant clinical signs and lesions, provide a diagnosis of ASF. However, it has a decreased sensitivity in subacute and chronic forms of ASF that could be related with the formation of antigen-antibody complexes in the tissues of infected pigs. This complexes block the interaction between the ASFV antigen and the conjugate (Sánchez-Vizcaíno, 2006 in OIE, 2012). The FAT can also help in the distinction of cytophatic effects produced by the ASF from other virus (OIE, 2012).

1.8.1.3 Antigen Elisa

This Enzyme-Linked Immunosorbent Assay (ELISA) method is used for detecting ASFV antigen, however it's only recommended for the acute forms of the ASF, since its sensitivity is believed to be lower than the PCR method. However this method has lower setting costs than the PCR methodology and it can be used in laboratories that have already ELISA technology (Oura et al., 2013; Steiger, Ackermann, Mettraux, & Kihm, 1992).

1.8.1.4 Polymerase-Chain Reaction (PCR)

The polymerase chain reaction (PCR) is a molecular method that allows the detection of specific DNA through the enzyme-based amplification of a short viral genome fragment. This method use primers from a highly conserved region of the genome, allowing detecting and identifying a wide range of isolates. The enzyme DNA polymerase generates multiple copies of the DNA that add deoxynucleotides (dNTPs) to a template piece of DNA (EURL-ASF, 2013b).

The real-time polymerase chain reaction (RT-PCR) is a modification of the conventional PCR, which allows the automated detection of the amplified product. This method is the gold standard diagnostic test for the detection of the ASFV genome, and it is used in all the OIE regional reference laboratories. It's a rapid method with sensitivity and specificity closed to 100%. It allows the viral detection before the onset of clinical signs in the infected animals (EURL-ASF, 2013c).

The sensitivity, specificity and quickness of these methods (conventional PCR and RT-PCR), allow control measures to be implemented in shorter times, when compared with other virus isolation methods (Oura et al., 2013).

1.8.2 Serologic tests

The serologic tests aim to the detection of specific antibodies in serum or in tissue samples (OIE, 2012). With no vaccine available, the detection of antibodies in the animals implies the presence of infection (Sánchez-Vizcaíno et al., 2015a). The antibodies develop in 7 to 10 days post-infection and persist for a long period of time. Despite the fact that antibodies induced by ASF infection don't have a protective effect, they are valuable tools of diagnostic, since they can provide valuable information about the endemic situation and the ASF carrying states (Sánchez-Vizcaíno & Mur, 2013 in Gallardo et al., 2015a).

The detection of antibodies have an epidemiological relevance since, in regions affected with ASF low virulent or avirulent strains and in the lack of evident clinical signs, the serological tests could be the only form of detecting the infected animals (OIE, 2012; Woźniakowski et al., 2016). This could be very important especially in endemic areas, where the confirmation of suspicious cases or outbreaks could be done using serologic test such as ELISA in combination with indirect fluorescent antibody test (IFA) or immunoblotting (Sánchez-Vizcaino et al., 2006 in OIE, 2012). Until recently, highly virulent strains, like the one circulating in the present European epidemic, were related with low percentage of antibody detection due to the early death before the development of detectable antibody response (EFSA, 2014; Guinat et al., 2014). However, in a recent published study, healthy antibody-positive animals (WB) were found in hunting bags in Estonia, which lead to a new scientific discussion (Nurmoja et al., 2017).

The ASF antibodies detection tests are, therefore, recommended for subacute and chronic forms as well as for large-scale tests and/or eradication programs (EURL-ASF, 2013e).

1.8.2.1 Enzyme- Linked Immunosorbent Assay (ELISA)

This ELISA is a method based on the formation of a complex between labelled antibodies and antigens. This labelling process with an enzyme allows that the resulting conjugated complex has an enzymatic and immunologic activity. The resulting compound is labelled with an enzyme and insolubilized, and therefore the antigen-antibody reaction could be immobilized (EURL-ASF, 2013d).

The recommended OIE' ELISA for ASF is an indirect ELISA assay method. The antigen is fixed in the plate and adding the samples of the suspicious animals with specific anti-ASF antibodies, it will be formed an ASFV antigen-antibody complex. After other several steps, whose description is out of the scope of this work, is added a marker to the complex to help identify the specific complexes in the sample (EURL-ASF, 2013d).

The European Reference Laboratory for ASF states that this method has a sensitivity of 95,8% and a specificity of 97,3%. Together with the fact that it can be used in a large number of samples in a short period of time, make this method the OIE recommended test for international trade (EURL-ASF, 2013d).

In addition an immunoblotting test, an indirect fluorescent antibody test (IFA) or an imunoperoxidase test can be performed in case of a doubtful result or a positive case in a poorly conserved sample (OIE, 2012).

1.8.2.2 Indirect fluorescent antibody test (IFA)

Indirect immunofluorescence test is briefly based on the use of glass slides carrying fixed ASFV infected cells (viral cells infected with adapted ASFV). Positive samples are revealed by UV light microscope upon incubation with anti-swine immunoglobulin sera labelled with fluorescein isothiocyanate (EURL-ASF, 2016).

This method is used as a confirmatory test, especially in ASF free areas with positive cases on the ELISA method and also for analysing samples from endemic areas with inconclusive results with other methods (OIE, 2012).

1.8.2.3 Immunoblotting

The immunoblotting is a fast and sensitive test for the detection and characterization of proteins (EURL-ASF, 2013d). This method is based in the antigen-antibody complex that is formed when ASF viral proteins that have been placed on antigen strips react with specific antibodies from samples (OIE, 2012).

This method is recommended by OIE as a confirmation test for samples that doubly tested positive with the ELISA method or for samples poorly preserved (OIE, 2012)

According to the European Reference Laboratory for ASF, this method has a sensitivity and specificity of 98% (EURL-ASF, 2013d).

1.9 ASF Epidemiology

1.9.1 Basic definitions

The OIE Terrestrial Animal Health Code (2012) (OIE, 2016) defines domestic pig as a permanently captive and farmed free-range pig, and distinguishes wild suids in WB and feral pigs. This latter can be defined as "*a domestic pig that is living in the wild, either having been released or having escaped from confinement*" (Jori & Bastos, 2009).

A suspicious case of ASF is defined as a "*pig or pig carcass exhibiting clinical symptoms or showing post mortem lesions or reactions to laboratory tests carried out in accordance with the diagnostic manual which indicate the possible presence of ASF*" (EU, 2002). The same document distinguishes a case from a suspected case of ASF which can be defined as the case " *in which clinical symptoms or post mortem lesions of African swine fever have been officially confirmed, or in which the presence of the disease has been officially confirmed as the result of a laboratory examination carried out in accordance with the diagnostic manual*" (EU, 2002).

1.9.2 OIE status

The OIE Terrestrial Animal Health Code (OIE, 2016), on its chapter 15, regarding ASF, states that in order to determine the ASF status of a country, zone or compartment, the following criteria should be fulfilled, both in DP and WB:

- *"ASF is notifiable in the whole country, and all clinical signs suggestive of ASF are subjected to appropriate field and laboratory investigations;*
- *"an ongoing awareness program is in place to encourage reporting of all cases suggestive of ASF";*
- *"The Veterinary Authority has current knowledge of, and authority over, all domestic pigs in the country, zone or compartment";*
- *"The Veterinary Authority has current knowledge about the species, population and habitat of wild pigs in the country or zone."*

A Country, zone or compartment can only be considered free of ASF if it's historically free of ASF, or if the free status resulted from an eradication programme (OIE, 2016).

The same code defines that, in order to be historically free of ASF, a country, zone or compartment should never had a case of ASF, the eradication has been achieved or the disease or infection has not occurred for at least 25 years. However, in order to be considered historically free, additional criteria should be met: the disease must have been considered notifiable in the last 10 years; an early detection must have been in place in all the relevant epidemiological species; measures to prevent the introduction must have been applied; no vaccination should be performed; no evidence of the disease or infection in wildlife (OIE, 2016).

When the free status is achieved through an eradication program, some criteria must be fulfill in order to the declared the free status of ASF (OIE, 2016):

- *" There has been no outbreak of ASF during the past three years; this period can be reduced to 12 months when there is no evidence of tick involvement in the epidemiology of the infection";*
- *"No evidence of ASFV infection has been found during the past 12 months";*
- *"Surveillance has been in place in domestic pigs for the past 12 months";*

The absence of ASF infection in any wild pig must also be demonstrated through surveillance in the country or zone declared as free. The follow requirements should also be met (OIE, 2016):

- *"There has been no clinical evidence, nor virological evidence of ASF in wild pigs during the past 12 months";*
- *"No seropositive wild pigs have been detected in the age class 6–12 months during the past 12 months";*

The recovery of the free status can occur in a country, zone or compartment if surveillance has been carried out with negative results through (OIE, 2016):

- "*Three months after the last case where a stamping-out policy is practiced and in the case where ticks are suspected to be involved in the epidemiology of the infection, followed by acaricide treatment and the use of sentinel pigs" or;*
- *"Where a stamping-out policy is not practise, the criteria to be follow is the same as the free status achieved through an eradication program";*
- *"Based on surveillance, ASF infection has been demonstrated not to be present in any wild pig population in the country or zone".*

1.9.3 History and current epidemiology of ASF in Africa

ASF was first described in the eastern African country of Kenya, in 1921 by the veterinary pathologist R. Eustace Montgomery (Plowright, 1986). This swine disease was described as an acute haemorrhagic fever with mortality rate close to 100%. It was also observed that the infection in DP occurred when these came in close contact with some wild suids species, especially warthogs (Reviewed by: Costard et al., 2009). These findings were soon confirmed in South Africa and Angola and, until today, it has been spreading through the African continent despite all the control and eradication efforts.

This disease has been considered endemic in the large regions of the sub-Saharan, with different epidemiological scenarios involving different hosts and reservoirs (Costard et al., 2009; Penrith & Vosloo, 2009). In the eastern and southern regions of Africa, ASF is known to be present in African wild hosts such as warthogs (*Phacochoerus aethiopicus*), bushpigs (*Potamochoerus porcus*) and soft ticks from the genus *Ornithodoros*, and spilling over to the DP. Beside this sylvatic cycle, the infection can also be due to a domestic cycle, occurring between infected and susceptible DP and transmitted through infected swill or as a result of animal movements (Reviewed by: Costard et al., 2009). In West Africa, where the warthog is not present, the domestic cycle seems to be the only responsible for the disease spread and persistence. The formal and informal trade of animals and direct contact are believed to be the major drivers of ASF spread and dissemination (Reviewed by :Costard et al., 2013).

1.9.4 History of ASF in Europe

The first outbreak of ASF in Europe occurred in Portugal in 1957, which match with the first report of the disease outside the African continent. The source of viral introduction was considered to be infected catering waste, which was used as feed in DP farms in the Lisbon region (Reviewed by:Costard et al., 2009). Despite this first introduction of the disease resulted in eradication, a second introduction in 1960 led to an endemic state in the Iberian Peninsula that lasted until mid 1990's (Costard et al., 2009; Sánchez-vizcaíno et al., 2006 in OIE, 2012). In the Iberian Peninsula, the eradication was only achieved after extensive and costly programmes funded by European Union. In this region ASF circulated in a cycle involving domestic pig, WB and the argasid tick *Ornithodoros erraticus* (Costard et al., 2013).

Portugal was considered to be free of ASF in 1993. A delimitated zone, mainly in Alentejo region, was adopted by the EU commission with imposed exportation restriction. In 1999 ASF re-emerged in a single outbreak having as the most probable cause the re-infection through vector borne transmission from an argasid tick (Boinas, Wilson, Hutchings, Martins, & Dixon, 2011; Sánchez-Vizcaíno et al., 2009). In Spain no outbreak has been reported since 1994, which has been recently confirmed by serological survey in WB in the Doñana National Park, one of the last ASF seropositive regions among the WB populations (Mur et al., 2012)

From the Iberian Peninsula, the disease spread also to the Caribbean region (Cuba (1971 to 1984), Dominican Republic (1978 to 1981) and Haiti (1979 to 1984)) and to South America. In Brazil, the disease was introduced in 1978 from Portugal or Spain, by transcontinental flights and/or animal products (Lyra, 2006 in Costard et al., 2013). The only exception was the italian island of Sardinia, where ASF remains endemic since its introduction in 1978 (Sánchez-vizcaíno et al., 2006 in OIE, 2012 ; Sánchez-Vizcaíno et al., 2009).

1.9.5 Current European epidemics

After a successful eradication effort in the Iberian Peninsula in the 1990's, ASF only remained endemic in the Sardinia Island. Here the disease circulates in a cycle that includes free ranging DP and WB without the existence of the soft tick acting as a vector or reservoir (Reviewed by: Sánchez-Vizcaíno et al., 2009).

Beside this endemic region of Sardinia, the disease re-emerged in Europe in 2007 in Georgia through the disposal of transcontinental infected waste in the Poti Port. The disease was reported in this country to OIE on 5 June 2007. Viral isolation and sequence analysis showed a close homology with the viral strain that circulated in that time in the southeast Africa. Only a small percentage of the outbreaks were notified and, since August 2007, there is no official reported outbreak (FAO EMPRES-i, 2017; Rowlands et al., 2008).

In late August 2007, ASF was reported in Armenia. Since then and until the end of the same year, 13 additional outbreaks were reported. Between December 2007 and 2010, no new cases were reported. However, in 2010 four new outbreaks were reported and, in 2011, eleven additional outbreaks occurred. In the WB populations of this country were declared three outbreaks that occurred in two distinct provinces. According to the Food and Agriculture Organization of United Nations (FAO), through its online Global Animal Disease Information System (FAO EMPRES-i, 2017), no new cases where in Armenia since 2011.

In November 2007, ASF arrived at the Russian Federation (RF). The first reported case was a WB found dead in the Shatoy'skoe Ushel'e region of the Chechen Republic (EFSA, 2014). After this introduction, the disease moved east spreading through WB populations. Between 2008 and 2010, an endemic area was established in southern and northern Caucasian federal states of RF. In this endemic area an epidemiological disease cycle between WB and free ranging DP was established. In the Caucasus region, DP are traditionally kept outdoors, which allowed the ASF transmission by direct contact between DP and WB in both directions. In three years 177 outbreaks were reported in the regions of Republic of North Ossetia, Chechen Republic, Republic of Kabardino-Balkaria, Krasnodarskii and Stravapol'skiy Kray (EFSA, 2014; Oganesyan et al., 2013). Between 2011 and 2013, while outbreaks were still occurring in the Caucasus districts, new cases arose in previously free territories. The disease started to move north towards the European part of the RF, reaching the regions surrounding Moscow, Tverskaya Oblast and Finland border, Leningradskaya Oblast and Murmanskaya Oblast (Oganesyan et al., 2013). Since 2012, a new endemic zone was established in the Tver region near Moscow, with a total of 34 outbreaks occurring between May 2011 and August 2012 (Sánchez-Vizcaíno et al., 2013). According to FAO (FAO EMPRES-i, 2017), since 2009, outbreaks have been notified in the RF both in DP and WB populations. The geographical leap that occurred from the first endemic zone to the north regions is thought to been consequence of anthropologic activities, such as the importation of infected meat or meat products (Beltrán-Alcrudo et al., 2009).

While ASF was spreading in the Russia Federation, new cases were also being notified in other countries, such as Azerbaijan, a country with a low density of DP, which are clustered in Christian communities. The only case reported in Azerbaijan involved the death and destruction of a total of 4,734 pigs. Suggestive cases of ASF were noticed, in 2013, through national media without official confirmation (EFSA, 2014; FAO EMPRES-i, 2017)

In the mid 2012, the first outbreak was notified in Ukraine at the Zaporozhye region, east of the Crimea Peninsula in a DP. The second case notified involved a WB, found in the riverside near the border with the RF. This is believed to have been the result of high hunting pressure near the border. Since the introduction until now, 202 outbreaks have been confirmed, with around 90% of the cases occurring in DP (FAO EMPRES-i, 2017).

In June 2013, ASF spread into Belarus. The first outbreak occurred in a DP farm in the western region of Grosno, which border with Poland. In July 2013, a second outbreak, also in DP, was reported in a region close to the Russian border (EFSA, 2014). Since then there has been no official notification of ASF outbreaks (FAO EMPRES-i, 2017).

On the 24th January 2014, the ASF genotype II that had been circulating in Eastern Europe entered in the EU territory in Lithuania. Since 1978 that ASF had been endemic in the Italian island of Sardinia but having ASFV genotype I as the responsible for the disease. The first two cases reported in Lithuania involved WB, located 40km from the border with Belarus. Genotype diagnosis found that the strain implicated in these cases had 100% homology with the case in the region of Grodno, Belarus. From January 2014 until July 2014, no new cases were reported. In July 2014, two new outbreaks were reported in areas located 160-180 km from the previous cases. Both outbreaks occurred in DP holdings, with one of them involving a large commercial farm that led to the death and/or destruction of almost 20,000 animals. The source of ASFV introduction in this case was related with human activities (EFSA, 2015). According to recent published data (EFSA 2017), until 2016, four clusters of cases were formed in Lithuania, with a limited annually spatial expansion. From the first outbreak until 17th of May 2017, 664 outbreaks were reported in OIE, with only 5,7% of them involving DP (FAO EMPRES-i, 2017).

Poland was the second EU member to declare a case of ASF in 14th of February 2014. The first case occurred in a WB found dead that tested positive for ASF DNA, 900 meters from the Belarus border. Three days later a new fresh WB carcass tested positive for ASFV three km from the Belarus border and 15km from the previous case. From mid February until mid April 2014, the Polish authorities actively search for ASF, sampling and testing 1,033 samples from DP and 2,868 samples from shot WB, and all of them were negative (EFSA, 2015). However, in May 2014, a new case was found in a WB carcass recovered from the river in the border of Poland with Belarus. In the following 12 months, 40 cases of positive ASF were confirmed within a radius of 30km from the second case found (Pejsak et al., 2014). The first case of ASF in a DP occurred in late July 2014 in a pig holding with 8 pigs. From the first case until the date of this review (17th of May, 2017) 222 outbreaks were confirmed in Poland, with nearly 90% of these confirmed outbreaks occurring in WB (FAO EMPRES-i, 2017). Since the epizootic began in Poland it had showed limited geographic spread in the WB populations, with most of the cases notified in the areas near the Belarus borders (EFSA, 2017). Results of a genetic analysis of the ASFV, together with epidemiological findings, confirm that the virus detected in both Poland and Lithuania was probably originated from Belarus (Gallardo et al., 2014).

The next EU member to be affected with a case of ASF was Latvia. On the 26th of June 2014, in the southern part of the country near the border with Belarus, three WB were found dead and tested positive for ASF. In the next day the first case in DP was also confirmed in a backyard farm with three pigs. The disease spread out fast, and one month later was detected in the central part of the country and in the regions bordering the RF (Ludza and Rezeknes). In late July a new WB case and a DP farm case with 58 pigs, were confirmed. In this latter, the source of introduction was considered to be contaminated grass used as a feed (EFSA, 2015). Since the first case until recently, it was suggested by EFSA (2017) the existence of four different clusters of cases. The epidemiological data suggest that the spread of ASF in WB population covered an area of almost 70% of the total territory of the country (EFSA, 2017). Until the date of this revision (17th of May, 2017) were confirmed a total of 1,184 outbreaks, with the majority of the cases involving WB (96.2%) and only a small percentage involving DP (3,8%). The outbreaks in DP, lead to the death and/or destruction of 1035 animals (FAO EMPRES-i, 2017).

Estonia was the latest EU country where ASF was introduced in the present epidemics. In September 2014, a WB piglet was found dead and tested positive for ASF, 6 km from the Latvia border. Five days after, a dead WB was also found 25 km from the previous outbreak, and tested positive for ASFV. In the same month, another WB was found dead and tested positive, 220 km from the previous case (EFSA, 2015). EFSA, in a scientific publication from 2017, considered the existence of four clusters of ASF cases in Estonia, including the cluster of two cases in WB on Saaremaa Island. In the particular case of this Island, human intervention was considered to be responsible for the ASF introduction. Due to the natural barriers between this island and the mainland, it was not possible the ASF introduction through WB migration from the mainland. Since the first case until the date of this review (17^{th} May, 2017) 1,054 outbreaks were confirmed in Estonia with 98.3% occurring in WB and only 18 outbreaks in DP. The DP outbreaks resulted in the death of 62 DP and in the destruction of 21,508 animals (FAO EMPRES-i, 2017).

According to EFSA (2017) the rate of geospatial spread among the EU countries affected with ASF was relatively slow (between 1 and 2 km/month). The same publication states that epidemiological data suggests that all the cases in WB in every country show the spatial-temporal pattern of small-scale epidemics.

The latest ASF spread dissemination in Europe occurred in October of 2016 in Moldavia. In this country two outbreaks of ASF in DP were confirmed in the northwest region of Edinet. No new cases were reported until March 2017, when two new outbreaks were confirmed with two weeks apart in DP. One of the outbreaks occurred in the Soroca region, which is close to the Ukranian border. All ASF outbreaks in Moldavia were reported to occur in DP, and led to the death and/or destruction of 29 animals in total (FAO EMPRES-i, 2017)

1.9.6 Transmission cycles

ASF is transmitted and maintained on a specific area through a transmission cycle that reflects the specific epidemiological context. This may vary according with the presence of different hosts, biological reservoir (ticks) or even with different commercial pig production systems (Costard et al., 2009; Sánchez-Vizcaíno et al., 2012).

1.9.6.1 Transmission cycle in Africa

Several authors have described a sylvatic cycle of ASF, which involves a transmission cycle between wild suids, *Ornithodoros* genus soft ticks and occasionally domestic pigs in southern and eastern Africa (Reviewed by: Costard et al., 2009, 2013).

This cycle involves the presence of warthog (Phacochoerus africanus), in which young suckling animals get infected by being parasitized by infected argasid ticks from the *Ornithodoros moubata* complex. This young warthogs produce a transitory viraemia that it's the source of infection to naïve ticks during the blood meals. This allows the virus to recirculate in the epidemiological context. The warthogs remain asymptomatically infected for life, and the virus can be detected in their lymphoid organs. The older warthogs do not develop enough viraemia to shed the virus, and the occasional transmission to domestic pigs occurs mediated by the biological vector, the argasid tick. In this case no horizontal or vertical transmission is observed between warthogs, and the maintenance of the infection in an area is only dependent on the soft ticks (Reviewed by: Costard et al., 2009, 2013; Sánchez-Vizcaíno, Mur, L., Bastos, A. D., & Penrith, M. L., 2015b).

In west and central parts of Africa, where evidence of the sylvatic cycle is lacking, the spread and transmission of ASFV occurs mainly through the movement of infected DP, infected swill and direct contact in free ranging systems (Reviewed by: Sánchez-Vizcaíno et al., 2015b). However, in some regions of Africa such as Malawi, only DP and the soft tick (*Ornithodoros porcinus*) have been implicated in the transmission cycle (Haresnape & Mamu, 1986).

1.9.6.2 Transmission cycle in Europe

Currently in Europe only DP and/or WB are implicated in the transmission cycle of the disease. However, in the past the soft tick (*Ornithodoros* genus) has been considered a reservoir of ASFV.

The disease spreads among DP and WB populations, which develop symptomatic clinical forms that may vary according to the viral strain present. The viral spread depends on several factors such as the presence of WB and/or DP, the DP production systems, biosecurity levels of farms, presence of arthropod vectors (*Ornithodoros* soft tick) and as stated before, the viral strain present in the specific region (Reviewed by: Costard et al., 2009).

As observed in the present European epidemic, WB and DP populations are differently impacted. While in some areas ASF spread occurred almost exclusively on DP population, in other areas, such as the Baltic countries and Poland, the disease is mainly spreading through WB populations (between 88,5% to 98,3% of the cases confirmed in WB) (FAO EMPRES-i, 2017). Although, in some other areas as it was observed in the northwest regions of Russia, the cases can occur in both populations and show a spatial correlation (Vergne, Gogin, & Pfeiffer, 2015)

In the domestic cycle present in Europe, the disease can spread through: direct contact between an infected animal and a susceptible animal; indirect contact transmission through contaminated fomites; through the consumption of infected meat, meat products or swill; and through vector borne transmission (Guinat et al., 2016b).

In the past ASF European spread that affected the Iberian Peninsula from the 60's to the 90's, the transmission cycle involved DP and WB, which have become contaminated mainly through the consumption of infected meat and swill. In this context the *Ornithodoros* *erraticus* soft tick was also implicated in the transmission, acting fundamentally as a reservoir (Reviewed by: Sánchez-Vizcaíno et al., 2012).

1.9.7 Prevention and control

Since neither vaccine nor a specific treatment for ASF is available, the disease management relies on sanitary/administrative measures based on legislation and governmental regulations. In light of this fact, prevention and control measures with appropriate contingency plans and surveillance programs are key points in ASF management (Gallardo et al., 2015a; Woźniakowski et al., 2016; Bellini et al., 2016). For appropriate response in case of an ASF outbreak, time is crucial, and therefore, the adequate early detection systems and contingency plans are corner stones of a rapid and effective response to new outbreaks (Sánchez-Vizcaíno et al., 2013).

The following description is based on the information gathered for the ASF context in the affected EU members and references EU legislation.

1.9.7.1 Preventive measures

The main preventive measure should be targeted to reduce the risk of ASF through the common transmission pathways (Bellini et al., 2016). According to Bellini et al. (2016) and DG SANCO (2013), the basic preventive measures to be taken are:

- In the risk areas, measures should be adopted to avoid or minimize the risk of transmission by direct contact between infected and susceptible animals. They should contemplate: physical barriers avoiding WB contact with DP; quarantine before introducing new animals to the holding and clinical surveillance; acquiring animals from non-infected farms;
- Since pig markets are locals where animals from different origins gather and contact, the pigs that have not been sold at the market shouldn't be reintroduced to the original herd before a quarantine protocol;
- Pig markets should be kept under supervision, and if disease is confirmed in an area, these markets should be suspended;
- Carcasses of DP and/or WB found dead should be notified and processed under official supervision. They also should be tested for ASFV, if decided by the competent authority;
- Awareness campaigns should be applied in order to inform pig farmers and operators of the pig sector. These campaigns should focus on the recognition of suspicious ASF

clinical signs, in order to enhance early detection and protective measures that could be applied to minimize the risk of ASF spread;
- Proper implementation of the swill feeding ban legislation;
- Visitor restriction, and enhanced biosecurity measures providing or requiring protective clothes and footwear;
- Properly disposing of manure, avoiding the spread of pig slurry in agricultural lands;

In the recent European context and due to the increasing importance of WB in the ASF geographical spread, WB hunting shouldn't be underestimated and preventive measures should be adopted to minimize the risk of spread and possible contamination to DP populations (Bellini et al., 2016).

1.9.7.2 Control

According to the Council Directive 2002/60/EC (EU, 2002) for EU members, when there is a suspected case of ASF in DP, it has to be reported immediately to the competent veterinary authority. When a suspicious case is confirmed in a DP farm, a protection zone of 3km is established, within a surveillance zone of 10km around the infected unit. The DP farms inside these zones are under enhanced surveillance and animal movements are restricted (Guinat et al., 2017).

When ASF is suspected or confirmed (including WB), the competent authorities from the affected EU member should establish an expert group that must provide assistance to the competent authority. This group must assist the demarcation of the infected area and the implementation of an eradication plan with surveillance of the infected area. In case of ASF outbreak in WB, the pig holdings in the infected area should also be subjected to a strict health monitoring, with the suspicious animals inspected and tested (DG SANCO, 2013).

1.9.7.3 Surveillance

Prevention and early detection of ASF play a major role in the control strategy, where surveillance can be seen as an indispensable tool (Bellini et al., 2016).

Surveillance, according to Thrusfield (2005), can be defined as "*the hability to document the occurrence of a disease with the goal of developing effective control and erradiaction strategies*", and thefore it's "*an essential part of disease contro*l". A more descriptive definition for surveillance (Hoinville et al., 2013) is "*The systematic (continuous or repeated) measurement, collection, collation, analysis, interpretation, and timely dissemination of animal-health and welfare data from defined populations*". The same authors add that "*These* *data are essential for describing health-hazard occurrence and to contribute to the planning, implementation, and evaluation of risk-mitigation actions*".

The data-collection methods can be used to classify surveillance, among many other possible different classifications. This traditional classification distinguish between passive and active surveillance. Passive surveillance can be, simply seen as a method where the provision of data depends on observer initated, such as relying on notification or existing data. In other hand, active surveillance can be defined as the method where the provision is investigator-initiated, implying an active search for data by the competent authorities (Thrusfield, 2005; Hoinville et al., 2013).

Passive surveillance in the ASF context is based on the investigation of sugestive clinical signs, high fatality rates in animal groups or by testing dead animals. In DP populations, this type of surveillance is dependent on early detection and awareness by the farmers. Therefore campaigns that help inform farmers to recognize the ASF signs are an important measure to be implementaded in high risk areas. In WB populations, passive surveillance relies on the search for ASFV viral DNA on WB found dead, since the observation of clinical signs on WB is unlikely. The notification of WB found dead has a pivotal role on the passive surveillance of ASF in WB populations (EFSA, 2010).

Active surveillance at the ASF context regarding DP, is based on a active search for sick animals and in the collection of samples for laboratory testing. This active search is mainly done by the clinical examinations of animals in pig holdings, usually in surveillance zones or considered at risk. Regarding the WB, active surveillance consist on the sampling and laboratory testing of shot animals in afected areas or at risk. Since there is a low probability of the direct observation of infected WB showing clinical signs, the active surveillance on this species relies on samples collected from shot animals (EFSA, 2010; DG SANCO, 2013)

Despite the advantages and disadvantages of each programs, both are usually necessary together, especially in response to an oubreak, were the combination of both will alow to maximize the probability of detection of new cases in a short period of time (EFSA, 2010).

According to EFSA on it's scientific review (2015), data from European ASF epidemics, especially on the EU affected members, proved that passive surveillance was more effective in detecting ASFV in infected WB and DP. This was concluded since all primary ASF outbreaks in DP holdings or WB had been found through passive surveillance. In the same document it was also stated that, according to the surveillance data, there is a higher probability of detecting ASFV in dead WB than in a live WB. The National Veterinary Institute in Sweden (SVA, 2015), state a similar conclusion, affirming that the current ASF strain circulating in the Baltic countries and Eastern Europe leads to acute clinical forms and high mortality. Therefore, early detection is most efficiently detected through clinical passive surveillance (SVA, 2015). In a recent published study (Guinat et al., 2017), a panel of ASF experts were consulted regarding the optimal surveillance strategies. In the opinion of these experts "*enhanced passive surveillance at hunted WB and WB carcasses and syndromic surveillance of pig mortality were regarded as the optimal suveillance strategies for detecting ASFV*".

The latest published surveillance data for ASF in the Baltic Countries (EFSA, 2017) point to an ASF's prevalence of 85.7% in WB found dead in Estonia, 78,2% in Latvia and 59,9% in Lithuania. In opposite, the ASF prevalence in WB hunted was 3% in Estonia, 2,1% in Latvia and 0,13% in Lithuania.

1.9.8 ASF and Sweden

Sweden is considered an ASF free country, since a positive case of ASF has never been reported in this country (SVA, 2014; FAO EMPRES-I, 2017). In Sweden it's mandatory to report any suspected case of an epizootic diseases, under the Swedish Act of Epizootic diseases, which includes ASF. This obligation is applied to animal owners, veterinarians and all the other relevant stakeholders. The suspected cases of ASF are investigated after expert consultation from the National Veterinary Institute (SVA) (SVA, 2015). All the reported cases of increased mortality or massive morbidity, along with clinical signs such as haemorrhagic disorders or reproductive failures, are considered suspicious of ASF until ruled out through investigation that could lead to the laboratory analysis of collected samples. Along with cases in DP, the "*Swedish hunters are encouraged to report all the findings of dead wild boar*" (SVA, 2015).

Due to the similar clinical presentation, all cases suspected of CSF are also analysed at the laboratory for ASFV DNA (SVA, 2015). The ASF surveillance is not only intended for early detection of a possible introduction, but also for documentation of the free status from ASF of the Swedish DP and WB populations (SVA, 2012).

In 2015, two clinical suspicious cases of ASF in DP were investigated and sampled, along with 15 samples analysed from WB found dead in the east and northeast part of Sweden. All cases were subjected to PCR, and all the samples were negative (SVA, 2015).

In 2014, only clinical suspicious cases of ASF in DP were investigated. Breeding sows with acute disease showing fever, discoloration of ears and mortality were investigated and samples collected for PCR. All the results came negative. In the same year, six cases of WB found dead in the south and southeast of Sweden were analysed for DNA of ASFV, and all the results came negative (SVA, 2014).

In 2013, three suspected cases of ASF in DP were investigated, and fourteen WB found dead, predominantly in the Southeast, were subjected to PCR testing with all the results negative (SVA, 2013)

In 2012, an active surveillance system for ASF along with the passive surveillance was still in practice. The sampling was part of surveillance carried out by the Swedish Animal Health Service along with other diseases (PRRS), and it was performed at the abattoir. The program analysed 20,146 sera from DP for antibodies with ELISA, and all the results were negative. Regarding passive surveillance, in 2012, five suspected cases were investigated, one of them in a WB with neurological signs. Following clinical examinations, samples were collected, and all the results came back negative. Samples from WB found dead were also analyzed for PCR, and all the results were negative (SVA, 2012).

In 2011, 2,262 samples were collected from DP at abattoirs and tested for antibodies for ASFV. All the results were negative. In the same year 15 suspected cases of ASF were investigated, mainly due to reproductive failures, and samples were collected for ASFV. All the results were negative (SVA, 2011).

2. OBJECTIVES

The aim of this study is to characterize the opportunities for spread of ASF at the interface between WB and DP. In specific, this study aimed to identify the possible transmission pathways between these two swine populations, producing a risk characterization of these pathways.

This study aimed at clarifying the role of WB in the hypothetical scenario of an ASF outbreak in the DP population in Sweden in order to inform the disease spread model currently being adapted in the country, as well as the actions to be considered for disease prevention and organisation of contingency plans. It will help to determine whether WB could serve only as a source of ASF infection (sporadic virus spill over events), or whether it could in fact contribute helping to maintain or even amplifying the spread among pig production holdings, and therefore impact the dynamics of disease spread during an DP outbreak.

3. MATERIALS AND METHODS

This study was divided into two steps. On the first step, transmission pathways between WB and DP were drawn without focus on any particular country. On a second step, each transmission pathway was detailed using event scenario trees and characterized into the Swedish reality. This method allowed identification of the risks for each transmission pathway, and specific information that needed to be collected to characterize the Swedish reality.

3.1 Transmission pathways at the interface WB-DP

A literature review was carried out in order to make an inventory of the known pathways for ASF transmission between WB and DP populations. This review targeted peer-reviewed literature on ASF, DP and WB.

The information collected from peer-review literature was complemented with four references, namely, three EFSA scientific opinions on ASF (EFSA 2010, 2014, 2015), and a risk profile on ASF published by the Finnish Food Safety Authority (EVIRA) (Oravainen et al., 2011).

The scientific document search was performed using the Scopus database (www.scopus.com). The search was restricted to documents written in English. After scoping exercises, a list of search terms was combined into the follow Boolean query: (“wild boar*” OR WB OR “wild pig*” OR “feral pig*”) AND (ASF OR “African swine fever”) AND (pig* OR swine OR pork OR porcine OR "*Sus scrofa domesticus*"). The terms were searched in the title, abstract and keywords sections.

The selection of the scientific documents was performed in two steps. In the first phase, the selection was based on title and abstract reading. Only ASF studies addressing both DP and WB were selected. The selected documents were subjected to full-text reading. In a second phase, references were retrieved from the bibliography of these articles, and subjected to a new abstract review. Only the documents considered relevant according to the previous criteria were added to the literature review.

The information collected was organized according to specific transmission pathways, and synthetized into a pictorial transmission map to facilitate expert review. Particular focus was given to organizing the current epidemiological knowledge about ASF according to the transmission modes identified, and highlighting similarity and differences in the transmission cycle in different regions where the disease was documented. This organization aimed also to identify knowledge gaps.

The validation of the conceptual structure of each transmission pathway was performed through extensive revision by the authors, and experts within the European Cooperation in Science and Technology (COST) funded action "*African Swine fever – recent research advances and strategies to combat the disease in Europe (ASF-STOP)*" both during a scientific conference, and through individual contact. The resulting schematic summary of the epidemiological knowledge available for each of the identified transmission pathways was meant to serve as a guide for the characterization of the risks of ASF spread between the DP and WB populations in any given country or region. To attend that purpose, an objective list of the epidemiological information needed to characterize each transmission pathway, in a particular region, was drawn from the information summarized for each pathway.

3.2 Risk Description and Swedish Risk Characterization

In each transmission pathway a detailed chain of events was drawn in order to assess and describe the relevant transmission risks. The risk description was performed using Event tree diagrams. "*Event trees offer a way to describe a sequence of probabilistic events, together with their probabilities and impacts. They are perhaps the most useful of all methods for depicting a probabilistic sequence, because they are very intuitive, the mathematics to combine the probabilities is simples and the diagram helps ensure the necessary discipline*" (Vose, 2008)

In each tree, the left-most event started with an infected WB or infectious secretions/excretion, and the chain of events needed to allow the exposure of a susceptible

DP, through that transmission pathway was detailed. In each node, a single event was assessed, and the information needed to characterize risks registered in a table.

The list of events and information needs drawn previously were used as a guide to collect targeted information regarding the Swedish reality, and perform a more detailed characterization of each transmission pathway.

The sources for characterization were primarily peer reviewed literature and available data from Swedish governmental and non-governmental institutions. Expert consultations were performed to complement the information collection. The information collection was organized into five distinct subjects: Pig production and organic pig production in Sweden; Biosecurity in pig farms; WB population; WB hunting; presence of *Ornithodoros* soft tick.

Geographical data handling and database analysis were performed using the statistical language R, through the interface RStudio (Version 0.99.489, 2009-2016). For the elaboration of maps it was used the software QGIS (Version 2.8.9-Wien).

As a geographic reference, it was used the Swedish regional level model of administration (länsstyrelserna), in which Sweden is divided in 21 county administrative boards (Government Offices of Sweden, 2017)

4. RESULTS

4.1 Transmission pathways at the interface WB-DP

4.1.1 Literature review process

The search returned 71 papers. The title and abstract review resulted in the selection of 31 references for full-text retrieval. Two of those articles could not be accessed. After full-text review of the available 29 references, 33 further abstracts were screened. From these latter 11 full-text articles were selected after careful abstract review. In total, 42 articles were included in the literature review.

4.1.2 Descriptive Results

The literature review led to the identification of five main ASF transmission pathways (Figure 1): direct contact, indirect contact, environmental contamination, infected swill, and vector-borne transmission. The information relevant to understand the opportunities for transmission between WB and DP populations, within each of these transmission pathways, is summarized below.

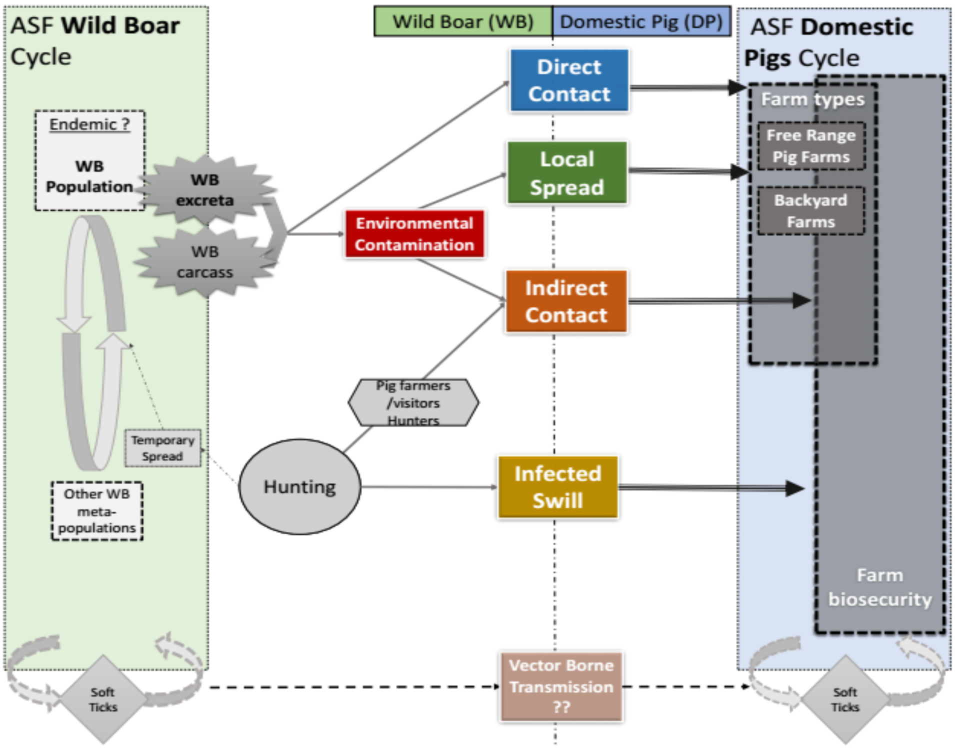

Figure 1 - Transmission pathways at the interface WB-DP

4.1.2.1 Direct Contact

Direct contact, in the specific context of this studied interface refers to the ASF transmission by the direct contact, namely oronasal, between a susceptible DP and an ASF infected WB or its secretions, excretions and/or blood.

Direct contact between infectious and susceptible DP is a proven mechanism of ASFV transmission (Guinat et al., 2016b). This transmission pathway is supported by the proven transmission susceptibility and efficiency of the ASFV by direct contact. It has been proven that WB are susceptible to the ASFV and develop disease in the case of infection, in particular with virus isolates from genotype II that circulate in the present European epidemics (Blome et al., 2012; Gabriel et al., 2011). The WB viral shedding when infected with ASF seems similar to the DP, which makes them able to transmit ASFV efficiently through direct contact with a susceptible DP (Gabriel et al., 2011; Jori & Bastos, 2009).

Laddomada et al. (1994), studying transmission in the endemic context of ASF in Sardinia, stated that the WB were less efficient in transmitting the infection than DP. However, Pietschmann et al. (2015), working with ASF viral isolates from the Armenia outbreaks, proved that even small doses of virus were efficient in transmitting ASFV, especially to weak or stunted animals.

Epidemiological data from the ASF epidemics in Europe reinforces the suggestion that WB may be efficient in transmitting ASF in this new context. In Poland, Śmietanka et al. (2016) affirms that in several outbreaks, the WB were the most likely source of infection for DP due to contact of both species, especially in poor biosecurity pig holdings. A similar role has been see in the north Caucasus, where the initial driven force of ASF spread is thought to be the contact between infected WB and free-ranging pigs (Gogin et al., 2013).

Possibility of transmission: It's a proven transmission pathway not only from DP to DP but also from WB to DP and vice-versa (Guinat et al., 2016b)

How could it happen: An ASF infected WB having contact with a susceptible DP, or a susceptible DP having contact with virulent excretions, secretions or blood from a WB (Guinat et al., 2016b).

What is needed to happen: It's necessary that both populations geographically overlap. The DP should have total or at least partial outdoors access to an area with infected WB (Gogin et al., 2013). The partial outdoor access should allow an effective contact between the susceptible DP and the infected WB or its excretions, secretions or blood.

Information needed:

- Characterization of the pig production practices and farms at risk;
- Outdoor pig productions units and their geographical presence relative to WB presence;
- Pig holdings that allow partial but effective contact with WB
- High risk seasons;
- Understand the size and location of the susceptible population of DP at risk of transmission by direct contact with a WB;
- Understand the nature and frequency of contact opportunities;
- Estimate the effective contact opportunities between WB and DP in a studied area.

4.1.2.2 Indirect Contact

Indirect contact, in the specific context of this work, refers to transmission between an ASF infected WB and a susceptible DP mediated by inorganic materials such as footwear, clothing and instruments carried by a human source.

In this transmission pathway, an inanimate carrier (fomite) Thrusfield (2005) is contaminated with ASFV from an infectious WB. The transmission of ASFV through a fomite between DP populations has been described in the scientific literature by some authors (Beltrán-Alcrudo et al., 2009; Mur et al., 2012; Williams, 2009; Sánchez-Vizcaíno et al., 2015a), but challenged by Guinat et al. (2016b), who stated that the infection of DP by contact with contaminated fomites has never been clearly demonstrated.

The proven viral resistance in the environment, especially at low temperatures and in organic materials, can facilitate the disease spread via fomites, especially if they contact with excretions, secretions and/or blood with high loads of virus (Davies et al., 2015; Gogin et al., 2013). However according to Pietschmann et al. (2015), even low viral doses are enough to infect susceptible animals, including DP, which highlights the potential for indirect transmission.

Since WB have a similar infectious dynamics and shedding to DP (Blome et al., 2012; Gabriel et al., 2011), it's plausible that they can play a similar role as the DP and be sources of environmental contamination and material contamination, and therefore transmission occur through indirect contact.

Possibility of transmission: this transmission pathway has been the subject of discussion, but is accepted by several authors as a possible way of ASF transmission. The role of WB has not been clearly assessed in this context, but it's plausible that it could have a similar role as the DP. In theory it is as likely as seen for the DP, since they are as susceptible and excrete in similar quantities as the DP (Blome et al., 2012; Gabriel et al., 2011). The question is then what are the opportunities for the contamination of people and vehicles from WB.

How could it happen: Through human contact with ASFV from an infected WB, after an accidental exposure or due to a high-risk contamination activity. The virus is then brought to a susceptible pig farm through contaminated clothes, footwear, instruments or even vehicles. The susceptible DP is consequently exposed when contacts with the contaminated source brought by a human. In this context, people can enter the farm due to its different profile in the farm, such as pig farm owner, employee, professional or a non-professional visitor. These profiles represent different contact opportunity with the susceptible DP as well different possible attitude towards the farm biosecurity measures. Pig farmers who also are WB hunters can represent a high risk for human introduced infections (Guinat et al., 2016b). This method of spread benefits but it's not dependent on proximity between the source and the susceptible host.

What is needed for this to happen: A person must have contact with infectious WB carcass, excretions, secretions and especially blood. Then the virus must survive in the contaminated materials and remains virulent. This contaminated person enters a pig farm and due to a breach on the pig farm biosecurity, the virus is carried by fomites and contacts with a susceptible DP.

Information needed

- Characterization of farm biosecurity level and practices to reduce the risk of human mediated virus introduction into pig farms, and the compliance of their implementation at a local or national level;
- Characterize pig production sectors that are at a higher risk of transmission through this pathway;
- Characterize the high-risk human activities that can directly relate with this transmission pathway;
- Characterize the biosecurity measures that in case of noncompliance could increase the opportunity for this transmission pathway;
- Assess expected viral resistance in the national or local weather conditions, and regarding different materials, organic or non-organic.

4.1.2.3 Infected Swill

In the specific context of this work, infected swill refers to the transmission route where a susceptible DP is exposed to the virus by being fed with uncooked or insufficiently cooked offal, meat or meat products from an infected WB. This route has been rarely assessed with the WB being the source of infection

Infected swill as a transmission route has had a very important role in ASF introduction and spread into new regions. It is considered to be the main reason for the historical first introduction in Portugal in 1957 and 1960, which led to the disease spread across Europe (Sánchez-Vizcaíno, Mur, & Martínez-López, 2013; Woźniakowski, Frączyk, Niemczuk, & Pejsak, 2016). It has been also implicated as the main reason of introduction in the current European epidemics, in the Poti port in Georgia in 2007 (Beltrán-Alcrudo et al., 2009; Sánchez-Vizcaíno et al., 2013). More recently it has been pointed as one of main reasons for the geographical spread of ASF in the RF (Gogin et al., 2013; Oganesyan et al., 2013).

This route occurs due to the fact that ASFV shows a good resistance to inactivation not only in the environment as well in pork products, which can persist for months due to is long persistence in pig tissues such as muscle, fat and bone marrow (Costard et al., 2013). No studies were found which directly address WB as the source of infected swill. However, as described before, this species presents a similar infection dynamic and shedding to the DP (Blome et al., 2012; Gabriel et al., 2011).

Possibility of transmission: It's considered to be the reason of ASF introduction in the current European epidemics in Georgia in 2007, and one of the main reasons for the spread of the disease into free regions in the RF (Beltrán-Alcrudo et al., 2009; Gogin et al., 2013; Oganesyan et al., 2013). However, the role of WB in this route was rarely assessed and quantified. Based on the susceptibility of the WB for the strains currently circulating in Europe and the same infection dynamics as seen for the DP, it can be assumed that the WB can be a source of infectious swill.

How could it happen: A susceptible DP is fed with uncooked or insufficiently cooked offal, meat or meat products from an infected WB.

What is needed for this to happen: Contaminated products containing meat, meat products or offal from an infected WB are given directly, or indirectly through swill waste resulting from human consumption of the contaminated products. The meat must be uncooked or insufficiently cooked for virus survival.

Information needed:

- Characterize feeding habits in different pig farms, focusing on the opportunities for illegal or accidental feeding of meat products.
- Information regarding hunting practices at a national level, offal disposal and WB meat consumption habits.

4.1.2.4 Environmental contamination / Local Spread

Environmental contamination/local spread in the specific context of this work refers to the all the transmission events that cannot be assessed or modelled individually. This includes for instance, airborne transmission and as well, virus carrying by mechanical vectors such as insects. These events can be translated into a risk of transmission that correlates inversely with the distance between the susceptible animals and the infected WB source (carcass, excretions, secretions and/or blood).

The role of the WB in the environmental contamination has been recently brought to discussion due to the rise of outbreak notifications in WB and the consequent increase of infectious WB carcass in the environment. This highlights the important role that this species has in the ASF spread at the present European epidemic (Guinat et al., 2014; Gavier-Widén et al., 2015). As previously stated, the proven resistance of the virus, especially at low temperatures and in organic materials, together with the high loads of virus present in WB excretions or secretions, facilitate the environmental spread and maintenance of the virus (Davies et al., 2015; Guinat et al., 2016b; Sánchez-Vizcaíno et al., 2013).

The contribution of insects in the transmission has been defended by some authors (Beltrán-Alcrudo et al., 2009; Gogin et al., 2013), while others state that there is no evidence that rodents, birds and other animals could assist in the transmission of ASFV (Sánchez-Vizcaino et al., 2015b). The only experimentally proven mechanical vector for ASFV is the *Stomoxys calcitrans* also known as the stable fly (Baldacchino et al., 2013). Airborne transmission has also been stated as plausible, but only over small distances (Wilkinson & Donaldson, 1977), while waterborne transmission was ruled out due to the virus dilution in the water (Beltrán-Alcrudo et al., 2009). Recently, the harvesting of fresh grass, which could be contaminated by WB secretions and excretions, has been implicated as the source of ASF infections in DP farms in Latvia (Guinat et al., 2016b).

Possibility of transmission: The events that can lead to transmission through this pathway are hard to model individually, and can be better represented as an overall risk function that is inversely correlated with the distance.

How could it happen: An infected WB shedding ASFV via excreta/secretions or an infected WB carcass is the source of virus in an area close to pig farm (Guinat et al., 2016b). The virus can be carried into the farm by distinct modes of local transmission, such as fresh grass harvested used for bedding, airborne, or mechanically via flies or other environmental agents.

What is needed for this to happen: The overlapping of infected WB population areas and susceptible DP farms and the presence of ASFV from an WB source, enhanced by the noncompliance to some biosecurity measures such as barriers to bird access, and vermin and rodent control programs in the pig farms.

Information needed:

- The geographic overlap of WB and DP populations;
- Information regarding the usage of fresh grass in pig farm daily practice at a local or national level;
- Characterization of farming systems and biosecurity practices that can increase the opportunities for local transmission events as described above.

4.1.2.5 Vector-Borne Transmission

In the specific context of this study, vector-borne transmission is the potential transmission pathway between an infected WB and a susceptible DP mediated by the agarsid soft tick *Ornithodoros* genus.

ASFV is the only DNA virus that can be efficiently transmitted by an arthropod vector, the argasid tick of the genus *Ornithodoros* (Reviewed by: Pietschmann et al., 2016). Other ticks have been subjected to study regarding ASFV transmission, however Carvalho Ferreira et al (2014) affirms that given to the lack of evidence for virus replication “in vitro” conditions in the ixodid ticks *Dermacentor reticulatus* e *Ixodes ricinus*, these ticks are unlikely to play any relevant role as biological vector for ASFV. The role of *Ornithodoros* soft tick has been historically described in Africa, mainly in East Africa involving the *Ornithodoros moubata* soft tick (Costard et al., 2013) and in past European epidemics involving *Ornithodoros erraticus*, with special focus on the Iberian Peninsula. In this latter region, it has played an important role as a reservoir of ASFV, delaying eradication despite the official eradication efforts (Costard et al., 2013).

In the recent European ASF epidemic, the role of *Ornithodoros* soft ticks has not been proved, despite the documented presence of some species of these ticks in the Caucasus regions (Beltrán-Alcrudo et al., 2009; Sánchez-Vízcaino et al., 2015). Since the demonstration that the current viral isolates circulating in the European continent replicate in the *Ornithodoros erraticus* soft tick (Diaz et al., 2012), some studies had tried to assess the presence of these soft ticks in free areas such as Germany (Pietschmann et al., 2016). With the disease increasingly affecting WB populations (Bosch et al., 2016), the potential role of soft ticks continues to be discussed, although it has never been proven that there is a relation between the *Ornithodoros* soft tick and the WB (EFSA, 2015; Boinas et al., 2014; Jori & Bastos, 2009).

Possibility of transmission: *Ornithodoros* soft ticks has been proven to be a vector for ASF in other epidemiological contexts, namely in Africa and in the Iberian Peninsula. Currently there is no scientific evidence of their implication in the present European epidemics, despite the presence of *Ornithodoros* soft ticks in the Caucasus regions, and demonstration that some of the current viral genotypes circulating could replicate in this soft tick.

How could it happen: A competent *Ornithodoros* soft tick has to feed on an infected WB. The soft tick must survive and the virus replicate in the soft tick. Afterwards the soft tick must bite a DP, and during the blood meal transmit ASFV.

What is needed for this to happen: The geographical overlap of the three populations (WB, DP, *Ornithodoros* soft tick) must occur. The soft tick must have a meal in the infected WB, and be able to replicate the virus. For completing the cycle the infected soft tick has to feed on a susceptible DP.

Information needed:

- Assess the presence of *Ornithodoros* soft tick, WB and susceptible DP populations in the considered region;
- Determine whether the soft ticks can infest both WB and DP in the region

4.2 Risk description and Swedish risk characterization.

4.2.1 Direct Contact

The scenario tree detailing the events that can lead to direct contact between a WB and a DP, drawn after reviewing the literature and supported by the Swedish characterization described in details below, is shown in Figure 2. An objective summary of the information needed to perform a risk characterization for any particular region or country, based on this event tree, is provided in Table 1. Table 1 also presents a summary of such a risk characterization carried out for Sweden.

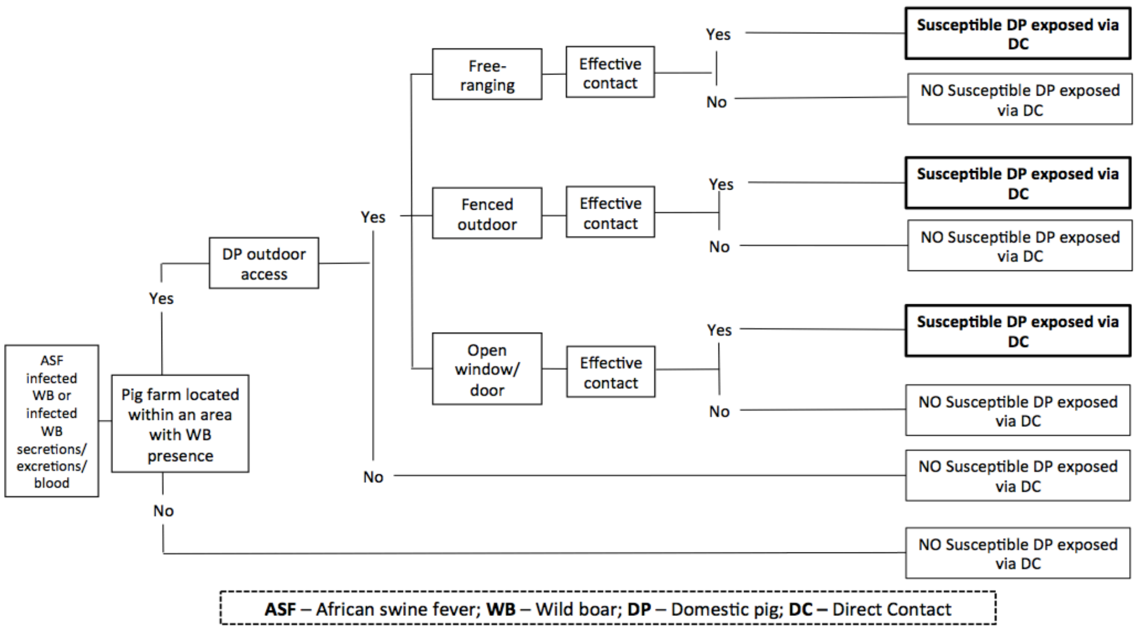

Figure 2 - Scenario Tree detailing the events that can lead to transmission of ASF through direct contact between WB and DP.

Event	Information needed	Scenario characterization in Sweden
Pig farm located within an area with WB presence	• Pig farms locations • WB population range	Pig farm density by county is shown in Figure 2. Estimated geographical distribution of WB based on reported hunting data in 2015, is shown in Figure 3.
DP with outdoor access	• Outdoor pig practices	Through a literature review and Swedish experts consultation, three main types of outdoor access were identified: Free ranging pigs; Fenced outdoors; Unheated stall that allow partial contact. Other types may need to be inventoried in specific regions. In Sweden, two main types of pig farms with outdoor

		access were identified: organic farming systems in which pigs have access to fenced outdoors areas during at least four continuous months of the year; and unheated buildings, “cold stalls”, which have an intermittent open door/window.
Free-ranging pigs	• Location of the free-ranging holdings, and characterization of their role in the industry	Currently in Sweden free ranging pig production (in non-fenced areas) is not practiced.
Pigs in fenced outdoor areas	• Pigs with outdoor access in fenced areas	Currently in Sweden, outdoor (fenced) pig production practice occurs in organic pig farming. The majority of the organic pig farms produce under the licensed label KRAV [Control Association for Alternative Cultivation], which demands at least four months of continuous outdoor grazing during warm months.
Cold stalls (Unheated buildings that allow partial outdoor access or contact)	• Pigs housed in cold stablls	Pigs housed in unheated buildings that allow contact with WB through an intermittent door or window. No data available on frequency of use of this specific type of holding, since there is no mandatory registration.
Effective Contact Probability	• Estimation on the probability of effective transmission for each of the types of contact identified	No specific event probabilities were estimated, as a risk assessment was out of the scope of this work.

Table 1 – List of information needed to characterize risks associated with the transmission of ASF between WB and DP through direct contact, as well as a description of these risks in Sweden.

Assuming that ASFV is circulating in the WB population in Sweden, the DP farms at risk of direct contact transmission are those who are situated in areas with WB presence. Pig farms are mainly concentrated in the South and Southwest of Sweden, with the counties north of Dalarna having the lowest densities, which represents less than one pig per km^2. In contrast the southern counties of Skåne and Halland show the national highest densities with over than eighteen pigs per km^2 as shown in figure 3 (SVA; 2015).

The geographic territory of the WB population in Sweden was estimated through the reports of WB shot. This data was available on the online hunting database from Swedish Association for Hunting and Wildlife Management (Svenska Jägareförbundet). The Swedish WB population is estimated to be present in a continuous territory that extends from the southern counties of Skåne and Blekinge until an undefined northern border formed by the Dalarna, Värmlands, Örebro and Gävleborg counties as shown in figure 3 and 4 (SVA, 2015; Swedish Association for Hunting and Wildlife Management, 2017).

In the last years, the WB populations showed a tendency to increase in number and expand towards northern regions. The real population dimension remains unclear. Some authors estimate a total number of 200 000 animals before the reproduction season of 2016 (SVA, 2015), however in the opinion of the wildlife and hunting expert Torsten Mörner, the real number is closer to 250 000 animals (personal communication, January 15, 2017).

In areas where the WB and DP populations overlap, outdoor access by DP represents the main risk for direct contact transmission at the WB-DP pig interface.

Based in the literature review and expert consult, three scenarios of outdoor access by pigs were identified: full outdoor access in non-fenced outdoor areas (free-ranging pig production), fenced outdoor access; partial outdoor access through specific pig housing with intermittent open windows/doors.

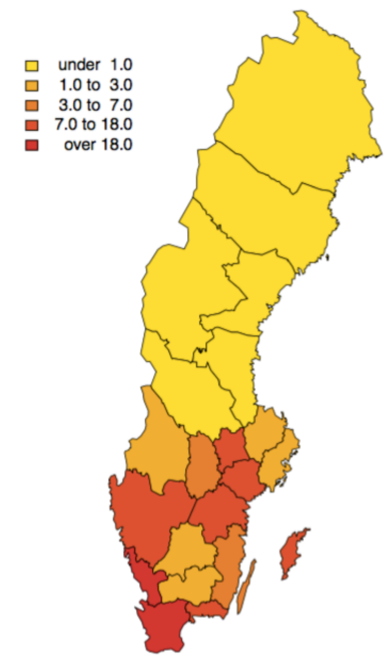

Figure 3: Number of pigs per km^2 in 21 Swedish counties as of June 2015.

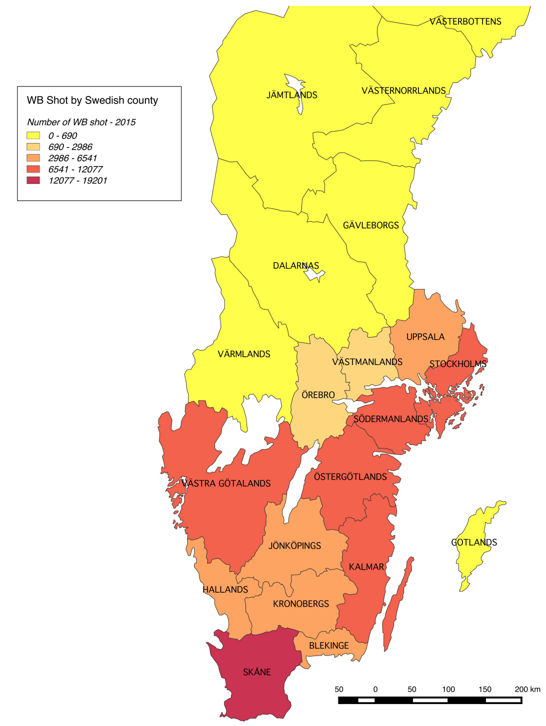

Figure 3 - "Number of pigs per Km^2 in 21 Swedish Counties as of June, 2015". Reproduced from SVA (2015)

Figure 4 – Number of WB shot by Swedish County. WB hunting data source: Swedish Association For Hunting and Wildlife Management, 2017; Swedish County data: Sverige Länsgränser (2017)

For the purpose of this work, free-ranging outdoor access was characterized as outdoor non-fenced access. This type of outdoors access is believed that is not practiced in Sweden. Only fenced outdoor access and partial outdoors access exist in the Swedish pig production, according with the experts consulted (Marie Söjlund and Per Wallgren, personal Communication, March 15, 2017). However, in this country the fenced outdoor pig practice occurs in the organic pig production context. Pigs can be produced organically through two different systems with complementary regulations. The most common is under the standards of the largest certifying body in Sweden (KRAV [Control Association for Alternative Cultivation]), which rules are stricter than the EU regulations for organic livestock farming. Along with other specific regulations, the KRAV reared pigs must have outdoors access in grazing and rooting on arable or woodland soil, for a period of at least 4 continuous months, usually the period between the beginnings of June until the late September (Wallander et al., 2016). EU regulations are considered less strict than KRAV, and according with this regulation, it's enough for pigs to have outdoor access in outside pens under a concrete slab (Früh, 2011; Jord på Trynet, 2017).

The official statistics of the Board of Agriculture in Sweden report 1,228 pig farms in 2015, 982 of which are fattening farms (Swedish Board of Agriculture, 2016b). Only around 30 fattening farms were reported to have KRAV certification in 2014 (Swedish Board of Agriculture, 2014). "Jord på Trynet", an association that represents 24 of these KRAV pig farms reports an annual slaughter of 24,000 organics pigs originated from these farms (Jord på Trynet, 2017). In other hand, the Swedish Board of Agriculture reported that in 2015, around 49,000 pig raised in organic system were slaughter in Sweden.

The "Jord på Trynet" farms that produced under KRAV label are located in 10 out of 21 Swedish counties: Dalarna, Uppland, Värmland, Väastmanlands, Södermanlands, Östergötlands, Hallands, Västra Götalands, Kalmar and Skåne. The counties with the highest number of KRAV farms register in this association are Uppland (7 farms), Skåne (6) and Västra Götalands (3). In all counties with active "Jord på Trynet" farms, were hunted WB, and therefore reported their presence. According with data available, 72,28% of the total 97, 262 shot WB in 2015, were hunted in the 10 counties were the "Jord på Trynet" krav pig farms are located (Jord på Trynet, 2017; Swedish Association for Hunting and Wildlife Management, 2017).

The partial outdoor access in the Swedish context is related with the use of specific unheated pig holdings for grouping housing. In this type of holding, frequently adapted barns, with wooden walls with slots that could allow partial contact between WB-DP. There is also the possibility existing of one or more walls with an intermittent window or door, which could be open during warmer months (Marie Söjlund and Per Wallgren, personal Communication, March 15, 2017).

Since this specific feature is not related with a specific type of production, the information regarding the use of this type of building is not recorded, and therefore was not possible to assess its implementation in Swedish pig production.

4.2.2 Indirect Contact

The event tree detailing the events that can lead to indirect contact between WB and DP, drawn after reviewing the literature and supported by the Swedish characterization described in details below is shown in Figure 5 – Indirect Contact Event Scenario Tree. An objective summary of the information needed to perform a risk characterization for any particular region or country, based on this event tree, is provided in Table 2. Table 2 - also presents a summary of such a risk characterization carried out for Sweden.

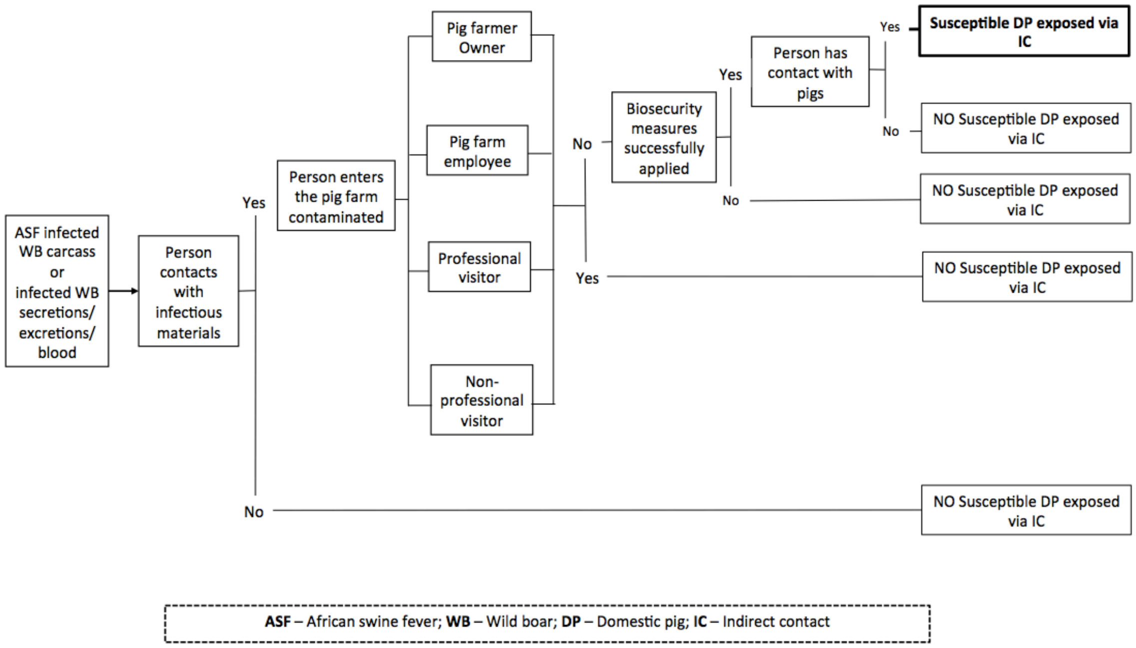

Figure 5 – Scenario Tree detailing the events that can lead to transmission of ASF through Indirect Contact between WB and DP.

Event	Information needed	Scenario characterization in Sweden
Person contacts with infectious materials	• Risk activities that can lead to human contamination with ASFV from infected WB	In Sweden, the identified risk activities were WB hunting and baiting stations (for hunting or crop protection purposes). Different human risk activities can be specified for different geographic contexts.
Person enters the pig farm contaminated	• Frequency of contact with DP according to the profile of the person who enters the pig farm.	In Sweden, four different profiles of pig farm visitors were identified: Pig farm owner; Pig farm employee; Professional visitor; Non-professional visitor. These profiles represent different roles within the farm, different biosecurity measures compliance and consequently different type of contacts with the animals, as detailed in the text.

Biosecurity measures applied successfully	• Compliance to effective biosecurity measures focused on minimizing the transmission through Indirect contact	Different visitor profiles will imply different compliance to the biosecurity measures that can impact the transmission through Indirect Contact. The main biosecurity measures considered were: Footwear and clothes changed before entering the farm; Properly sanitization of high activity tools; Avoid the sharing of high risk tools with pig farm practicing; Not allowing personal vehicles to enter the pig farm.
Person has contact with the pigs	• Contact rate between the person who enters the farm and the pigs (according to the different profiles)	Each profile will have a different role in the farm, and consequently a different likelihood of contacting pigs. This different likelihood of contact will impact in the transmission opportunity for indirect contact, according with the profile analysed.

Table 2 - List of information needed to characterize risks associated with the transmission of ASF between WB and DP through indirect contact, as well as a description of these risks in Sweden.

Indirect contact is defined in the context of this work as the ASF transmission between an infected WB and a susceptible DP mediated by inorganic materials such as footwear, clothing and instruments carried by a human source.

While an accidental exposure can be possible, two main risk activities were identified in Sweden which can enhance the human exposure to the virus: WB hunting and WB baiting.

The first human risk activity identified was WB hunting. It's a popular activity in Sweden with an increasing importance. Is estimated to exist 300,000 registered hunters in Sweden (Swedish Association for Hunting and Wildlife Management, 2016), however this number doesn't allow distinguishing WB hunters from the total number of registered hunters. Since 2013, the number of shot WB has increased, with 97,626 shot WB in 2015 (Swedish Association for Hunting and Wildlife Management, 2017). Blood, nasal discharge, urine and faeces are sources of ASFV when a WB is infected with ASF (Davies et al., 2015; EFSA, 2009; Gabriel et al., 2011; Pietschmann et al., 2015). Hunters can get contaminated during activities leading to the exposure to these WB infectious excretions, secretions and/or blood. Contamination of clothes, instruments and vehicles could happen not only through carcass handling and manipulation (removing skin and dismantling carcass parts) but also by stepping into WB hunting grounds.

The second human risk activity identified is WB baiting (Figure 6). The baiting stations are feeding places with the goal of attracting WB for hunting purposes or by distracting them from crop fields (Lemel, 1999 in Magnusson, 2010). These stations are not subject to an official registration and their exact location and total number are therefore unknown (Torsten

Mörner (personal communication, January 15, 2017). As an attraction site, WB baiting stations could gather animals from different family groups and enhance the contact between them. In a hypothetical ASF epidemic in Swedish WB populations, these sites could harbour high loads of ASFV in the environment and therefore be, not only, a source of human contamination but also a source of infection for naïve WB groups.

Assuming that a person can be contaminated, in order to transmit ASFV to a susceptible DP, it must enter the pig farm and contact with the susceptible DP. Four different profiles of persons that enter the farm were considered: Pig farm owner; Pig farm employee; Professional visitor; Non-Professional visitor. Each profile translates a different compliance to biosecurity measures and a different contact opportunity with the susceptible animals.

Figure 6 - Wild boar baiting station (Picture kindly supplied by
Source: Torsten Mörner)

Pig farm owner and employee can have a potential role in this transmission pathway, especially due to their higher likelihood of contacting with the susceptible DP. Pig farmers and farm employees that are also WB hunters are a particular high-risk group for indirect contact transmission (Guinat et al., 2016b). No specific publication assessing the frequency of daily contact between farmers or employees and the pigs in the Swedish context is available to our best knowledge, but it's plausible that would be among the highest when compared with the other profiles. Not all farmers and employees represent the same risk for ASF introduction. Their compliance to biosecurity can depend on their personal practice but also in the type of production unit that they are linked. "Small hobby" (non-professional) and small mixed species farmers could be at a higher risk for this transmission pathway, since it's recognize that these types of farms are the ones with lower biosecurity levels in Sweden (Nöremark, Frössling, & Lewerin, 2010; Nöremark, Frössling, & Lewerin, 2013).

Professional visitors are all the visitors with a professional purpose such as veterinarians, electricians, salesmen's, transport drivers, and they are more difficult to restrict when compared with non-professional visits (Nöremark et al., 2013). Once again, it's plausible that not all professional visitors pose the same risk of introduction the virus. Is likely that different professional categories have different compliances toward biosecurity measures as well, different contact rates with the animals. An example is the higher reported use of protective clothes by veterinarians when compared with salesmen and repairmen (Nöremark & Sternberg-Lewerin, 2014). In a recent study including farms with different species of livestock, pig farms were reported to have less than 1 professional visit by day, and that this number fluctuate little during the year. One possible explanation suggested by the authors, is the fact that Swedish pig farmers are allowed to keep some antibiotics and perform a first line of treatment, which could result in a lower number of veterinarian visits (Nöremark et al., 2013).

Swedish studies revealed that non-professional visitors, such as tourist, neighbours and others, were more frequent than professional visitors in pig farms, but their frequency was still minor than one visitor per day (Nöremark et al., 2013). The neighbour was the most frequent non-professional visitor reported in pig farms. In the same study, it was reported that, in small mixed farms (farms including more animal species besides pigs) the number of non-professional visits surpass a lot the number of professional visits, mainly in the summer.

If a person in any of the previously discussed profiles has contact with infectious materials from WB, the virus can be carried in clothes and footwear, instruments and vehicles into the farm. If these fomites enter a pig farm and have contact with a susceptible DP, transmission can occur through the indirect contact pathway. While the general level of biosecurity of a farm is a good indicator, there are some specific biosecurity measures that can have a direct impact in this transmission pathway such as: footwear and clothes changed before entering the farm; proper sanitization of high activity tools; avoiding the sharing of high risk tools with pig farm practicing; not allowing personal vehicles to enter the pig farm.

In Sweden the pig farms implement, in general, higher biosecurity measures regarding protective clothes and footwear when compared with other types of livestock farms. Nöremark et al. (2010) in a study that assessed biosecurity routines through farmers' questionnaires stated: "*74% of the pig farmers replied that they require visitors to wear protective clothing always or almost always*" Other study also in pig farms, point that "*90% of herds provided visitors with boots and clothing*" (Backhans, Sjölund, Lindberg, &

Emanuelson, 2015). However, conclusions regarding the implementation of these measures are hard to draw, since there is a great variation on biosecurity measures between small and large pig farms (Nöremark et al., 2010).

Contaminated instruments, such as knives, chains and other hunting or baiting tools, can pose a threat of transmission. According with the opinion of the wildlife and hunting expert Torsten Mörner (personal communication, January 15, 2017), in rural areas hunters usually carry one or several pocket knives that are used for different purposes besides hunting. The absence of cleaning and disinfection routines after hunting practices, along with the introduction in farms of these tools through sharing habits can drive to a transmission opportunity. Sharing tools is not a rare habit among farmers in Sweden. One Swedish study targeting pig farmers through questionnaire reported that 57% of the farms shared equipment with other farms (Nöremark et al., 2010).

The contamination of hunting vehicles also needs to be considered. According to the opinion of the wildlife and hunting expert Torsten Mörner (personal communication, January 15, 2017), Swedish hunters usually don't have a specific vehicle for hunting and often it's their own personal car that transports the WB carcasses. There are no specific regulations for cleaning and disinfection of hunting vehicles, and no studies were found investigating the practice.

4.2.3 Infected Swill

The event tree detailing the events that can lead to ASF exposure of a DP by infected swill from a hunted WB, drawn after reviewing the literature and supported by the Swedish characterization described in details below is shown in Figure 7. An objective summary of the information needed to perform a risk characterization for any particular region or country, based on this event tree, is provided in Table 3. Table 3 also presents a summary of such a risk characterization carried out for Sweden.

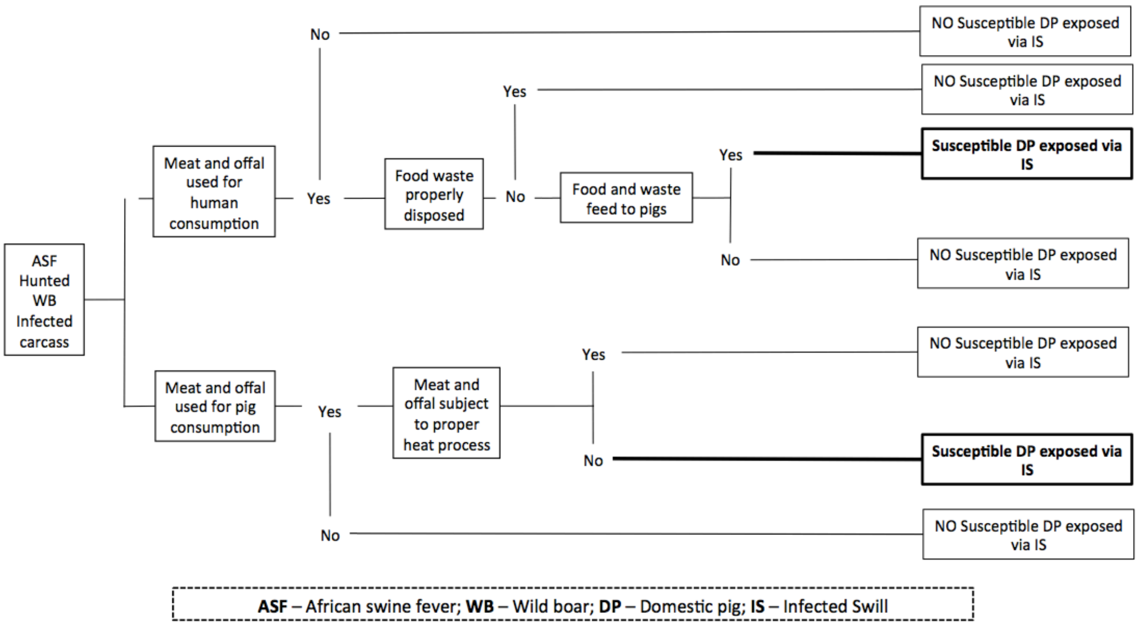

Figure 7 - Scenario Tree detailing the events that can lead to transmission of ASF through infected swill transmission pathway between WB and DP.

Event	Information needed	Scenario characterization in Sweden
Meat and offal used for human consumption	• Destination of the meat from the WB hunting	Meat from hunted WB is intended for human consumption (Torsten Mörner, personal communication, January 15, 2017), but no information was found regarding disposable of non-edible parts.
Food waste properly disposed	• Common practices on waste from animal products	No specific information was found regarding disposal of meat products.
Food and waste feed to pigs	• Compliance of EU swill feeding ban among pig farmers	Pig swill feeding is banned in Europe, however (Nöremark et al, 2009), report that small herds can be less aware of official communications..
Meat and offal used for pig consumption	• The use of hunted WB meat as pig swill feeding	There is no data regarding pig feeding directly with meat and offal from hunted WB.
Meat and offal subject to properly heat process	• Use of heating process before using animal waste to swill feeding.	There is no data regarding swill feeding from waste or WB meat, nor heating process before this practice

Table 3 – List of information needed to characterize risks associated with the transmission of ASF between WB and DP through infected swill, as well as a description of these risks in Sweden.

In the specific context of this work, this transmission pathway begins with a hunted WB carcass and ends with the exposure of a susceptible DP through feeding of infected swill or meat from the infected WB.

This transmission pathway can occur through two distinct paths: WB meat that is intended to human consumption with the exposure of a susceptible DP through human waste containing infected WB remains; or the exposure of a susceptible DP by being directly fed with uncooked or improperly cooked infected WB meat or offal.

In Sweden, hunted WB are mainly destined for human consumption. The meat is usually consumed as cooked meat, and products (sausages, etc.) derived from hunted WB are rare, according with opinion of the wildlife and hunting expert Torsten Mörner (personal communication, January 15, 2017).

WB meat consumption in Sweden is mostly associated with hunting, despite some importation of WB meat from other countries (Wiklund & Malmfors, 2014). From the hunted animal until the final edible meat, several steps of skinning and carcass preparations must be done. The majority of hunted WB in Sweden (90%) are yearlings, weighing on average 60 kg. After skinning and some preparation, the carcass weights around 55% of the live weight (Torsten Mörner, personal communication, January 15, 2017). The edible meat proportion is around 55 to 65% of the weight after the previous steps (Wiklund & Malmfors, 2014), which represents around 18 to 21kg of meat. In the last three years, WB hunting seems to maintain an increasing trend, with 97 626 hunted WB reported in 2015. This represents roughly 1.8 to 2.1 tons of meat, and 3.8 to 4.2 tons of non-edible parts.

When the hunted WB meat is destined to human consumption, some of this meat after being consumed or after the preparation process could end on waste. According to Swedish Environmental Protection Agency (Naturvåardsverket) (2014), in 2012 were generated 771 000 tons of food waste from households, however no specific data regarding the amount of meat, or in particular WB meat that end up in waste was found.

Sweden as a EU member is obligated to apply the EU legislation bans the use of swill in the pig feed (Bellini et al., 2016). It's hard to assess the compliance of this practice in Sweden, since there is no data available. The only information available by Nöremark et al. (2009) reported that small herds can be less aware of official communications and therefore can be more likely to practice swill feeding and have unregistered animals, however, currently due to the lack of information this idea cannot be confirmed. The experts consulted have the same opinion, Per Wallgren and Marie Sjölund (personal communication, March 15, 2017), in which Sweden could possible have small-scale backyard pig farms where can exist unregistered pigs. However, these units are not part of the production cycle that includes commercial pig farms, representing "dead-ends" in the network, since they usually buy pigs from commercial farms, but grow them for self-consumption, as abattoirs will not receive unregistered animals. Among these farms, the compliance to the swill feed ban could be lower than in commercial farms.

It wasn't found any data or information regarding DP being fed directly with meat or offal from hunted WB in Sweden. Neither was found any information regarding thermal process of these products in this context.

4.2.4 Environmental Contamination / Local Spread

The scenario tree detailing the events that can lead to ASF transmission between WB and DP by environmental contamination/ local spread, drawn after reviewing the literature and supported by the Swedish characterization described in details below is shown in Figure 8.

An objective summary of the information needed to perform a risk characterization for any particular region or country, based on this event tree, is provided in Table 4. Table 4 also presents a summary of such a risk characterization carried out for Sweden.

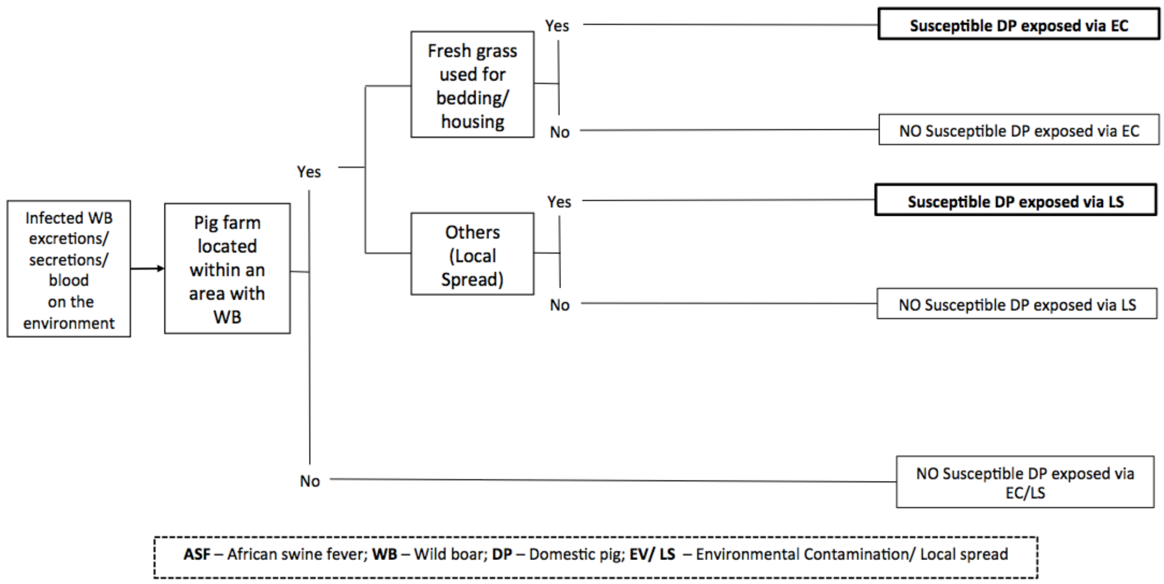

Figure 8 - Scenario Tree detailing the events that can lead to transmission of ASF through environmental contamination /local spread between WB and DP.

Event	Information needed	Scenario characterization in Sweden
Pig farm located within an area with WB presence	• Pig farms locations • WB population locations	Swedish pig farms density by county is available in the figure 2. Estimated geographical WB presence based on reported hunting data (2015) is presented in figure 4.

Fresh grass used for bedding/ housing	• Frequency of use of fresh grass to bedding or housing in pig farms	There is no data regarding the use of fresh harvested grass for bedding or housing in Swedish pig farms, however according to (Wallgren, Westin, & Gunnarsson, 2016) a very high percentage of pig farms use straw as environmental enrichment.
Other (Local Spread)	• Estimation of the probability of local spread transmission based on distance and farms biosecurity.	Probabilities estimation is out of the scope of this work. However, the possible risk contributors in Sweden are: presence of *Stomoxys calcitrans* “stable fly” and low biosecurity levels in “small hobby” (non-professional) and small mixed species Swedish pig farms.

Table 4 – List of information needed to characterize risks associated with the transmission of ASF between WB and DP through environmental contamination / local spread, as well as a description of these risks in Sweden.

Since for this pathway distance is an important risk factor, it has to be considered that geographical overlap between WB populations and DP populations must occur. As described in the scenario characterization for direct contact transmission pathway, all or almost all, pig farms could be assumed to be located in a areas with WB presence.

Assuming the scenario of ASF spread among WB populations in Sweden hunting activities with carcass and offal disposal can contribute for environmental contamination and consequently increase the risk of transmission through this transmission pathway. According to the opinion of wildlife and hunting expert Torsten Mörner (personal communication, January 15, 2017), it’s possible that offal from hunted WB can be removed and left in the hunting grounds. This practice could lead to persistence of the virus in the environment (Guberti, 2016) and contribute to the reinfection of naïve WB populations and to the introduction of the virus by environmental contamination/local spread. Besides hunting, also WB carcasses resulting from the natural infection can perpetuate the permanence of the virus in thee environment (Guberti, 2016; Probst, Globig, Knoll, Conraths, & Depner, 2017) and therefore contribute as source of ASFV in the context of this transmission pathway.

In this pathway were considered all the transmission events that couldn’t be individually assessed such as airborne, transmission mediated by birds, insects or rodents. In this context is worth mention that the only experimentally proved mechanical vector for ASFV, the *Stomoxys calcitrans* is present in the Swedish territory (Baldacchino et al., 2013; Vaduva, 2015).

The only event individually considered was the fresh grass contaminated with ASFV from a WB source. Its importance has been highlighted in the ASF literature, since it’s described as a possible reason for outbreaks that occurred in DP farms in Latvia (Guinat et al., 2016b).

However in Swedish pig farms, fresh grass is not used for bedding, according to the expert opinion of Per Wallgren and Marie Sjölund (personal communication, March 15, 2017). Straw is widely used as a strategy for environmental enrichment for preventing tail biting in Swedish pig farms, since tail docking is not allowed (Federation of Swedish Farmers, 2015). A recent Swedish study in pig farms pointed that 99% of the farms included in study reported the usage of straw (Wallgren et al., 2016). Due to environmental resistance of the ASFV in organic materials, it can't be ruled out that ASFV virus could still be viable in straw.

All the other forms of transmission that couldn't be individual assessed and are based on distance were grouped into the box "Others (Local Spread)". Here are grouped events such as airborne transmission, and mechanical transmission through rodents, scavenger birds or flies. In this context is worth mention that the only experimentally proved mechanical vector for ASFV, the *Stomoxys calcitrans* is present in the Swedish territory (Baldacchino et al., 2013; Vaduva, 2015).

The major key factors for local spread are distance, farm biosecurity and housing type. The closer the farm is from the infection source (WB carcasses or contaminated sites), the higher is the likelihood of transmission. The higher the probability of local transmission, the more important becomes the proper adoption of biosecurity measures. Insect and rodent control programs and physical barriers for birds and rodents are examples of biosecurity measures that could impact the transmission likelihood for local spread. Different housing types can offer different challenges to local spread prevention. "Cold stalls" and outdoor fenced production systems are examples of systems more exposed to transmission opportunities by birds, rodents, wind and flies. In contrast, commercial farms with exclusive indoor practice and controlled indoor environment are less vulnerable to local spread.

5. DISCUSSION

The role of WB in the ASF spread has gained more relevance since the introduction of the disease in the Baltic countries and Poland (Bosch et al., 2016). However, this role is still not fully understood, namely in the impact that WB may have on the transmission of the disease in DP populations. In this work is presented a literature review, which supported a thorough characterization of risks for each of five transmission pathways, discussed below.

ASF transmission by direct contact can occur if two conditions are fulfilled: both WB and DP populations are present in the same geographic area, and there's an opportunity for contact between them. In Sweden the first condition seems to be true for all areas in which WB are present, with the WB and DP populations overlapping.

The opportunities for direct contact are then related to the conditions of animal housing. The highest risk is associated with animals that have outdoor access. In Sweden this type of farming is very limited – different sources consulted showed different numbers, but the maximum reported number of farms found was 30 (Wallander et al., 2016), which represents approximately 2% of the pig farms in Sweden (Swedish Board of Agriculture, 2016). This is small when compared with other EU countries such as Italy, Germany, Denmark and UK (Früh et al., 2014). Moreover, outdoor access is limited to 3-4 months a year.

A second housing system that could provide opportunity for direct contact are the "cold stalls", in which the animals are kept indoors, but houses have windows. The chance of direct contact through the openings in these types of stalls is very low in the opinion of the experts consulted, but it cannot be excluded. Further studies are needed in order to estimate the chance of effective contact between WB and DP in this type of housing, and in particular the extent to which this type of housing is used. No registers seem to exist regarding the number of farms using this type of housing.

In summary, if ASF virus would circulate in the WB population in Sweden, the risk of transmission to DP through direct contact would be concentrated in a small number of organic farms, in a small period of the year, usually from the beginning of June until the end of September (Wallander et al., 2016). In such a scenario extra precautions could be needed to avoid exposure of DP housed in cold stalls, at least until further studies can assess the real risk of virus transmission from WB.

The indirect contact transmission pathway can occur when a human is contaminated with ASF from a WB source, and then enters a DP farm introducing the virus to a susceptible DP through a fomite. The risk of transmission is related with high-risk contamination activities (WB hunting and baiting), the type of human profile that enters in a farm and the farm biosecurity level.

Due to use of hunting as a WB population control strategy in the Baltic countries and Eastern Europe (EFSA, 2015), its role in the environmental contamination and fomite transmission has been subject to discussion. However, more information is needed in order to assess the real role of WB hunting in Sweden as a source of ASFV in the context of this pathway.

It's recognized that not every person that enters a DP farm pose the same risk of introducing ASFV, once again an assessment of the risk of contact and transmission of the different profiles is needed.

Farm biosecurity is a key risk factor in the probability of this transmission pathway. The biosecurity of the Swedish pig farms is recognized to be high, not only when compared with other livestock farms in Sweden (Nöremark, Frössling, & Lewerin, 2013) as well when compared with other countries, especially in external biosecurity measures (Postma et al., 2016). The population at a higher risk, the "small hobby" pig farms linked with lower biosecurity standards in Sweden (Nöremark et al., 2009), represent less than 1% of the total pig population (EUROSTAT, 2014). Moreover, the pig production experts consulted in this work have highlighted that "hobby farms" do no sell or even send animals to slaughter in commercial establishments, and therefore are not believed to be able to contribute to a cycle of virus transmission within the DP production system.

The transmission through WB infected swill could occur if a susceptible DP is exposed to the virus by being fed with uncooked or insufficiently cooked offal, meat or meat products from an infected WB. The key risk factor is the compliance to the swill feed ban by the pig farmers, which seems to be very high in Sweden. Low disease awareness and low level of overall biosecurity were associated with small scale farms (Nöremark et al., 2009), but a direct investigation of feeding practices was not reported, and as stated, "hobby farmers" represent a minimal part of the DP population in Sweden, and are believe to be "end nodes" within the network, receiving but not sending pigs to other farms. Therefore the risk of transmission due to infected swill originated from infected WB is likely to be low, in contrast to the RF, where the swill feeding with infected pork products has been recognized to be a driven source in low biosecurity backyard farms (Khomenko et al., 2013). It is worth noticing, however, that a large volume of hunted WB has been reported in Sweden (Swedish Association for Hunting and Wildlife Management, 2017), and further work should address the destination of meat and offal, to ensure that it could not end as DP feed.

In the environmental contamination/local spread pathway the risk of transmission is inversely related with the distance between the source of the ASFV and the susceptible DP, with the farm level of biosecurity and with the farm type. ASF, to the best of the authors' knowledge, has never been subject to a distance-based transmission assessment unlike other infectious diseases such as Foot-and-mouth disease and PRRS virus (Dee, Otake, Oliveira, & Deen, 2009; Garner & Cannon, 1995), despite being recognized that airborne dispersion of the ASFV could occur under experimental conditions (de Carvalho Ferreira, Weesendorp, Quak, Stegeman, & Loeffen, 2013b). Besides farm biosecurity, the farm type can enhance the risk of transmission, therefore it's plausible that outdoor pig production systems, such as organic, can be at a higher risk. This type of pig production system is also associated with a higher probability of the transmission of zoonotic agents, due to the fact that these pigs are more susceptible to contact with wild animals, namely rodents and birds (Salajpal, Karolyi, & Luković, 2013).

In other countries, the use of fresh grass to feed pigs has been implicated as a risk factor in the transmission of ASF (Oļševskis et al., 2016). Feeding grass to pigs was not found to be a practice used in Sweden, however the use of straw bedding is common. No studies were found addressing the risk of virus survival in dried straw, but further assessments would be needed to rule out the possibility of environmental contamination.

The role of vectors in the transmission of ASFV from WB to DP has not been confirmed (EFSA, 2010), and so far it is believed to not play a role in the transmission in the Baltic countries (EFSA, 2015). Even if transmission through soft ticks were to play a role, in the same way that it does in areas where the wild host is not a wild boar but the warthog, the presence of the *Ornithodoros* soft tick was never reported in Sweden. Vector-borne transmission is therefore not considered relevant when trying to assess the risk of ASF transmission from possibly infected WB to DP in Sweden.

This work provides a thorough review of information relevant to understand the opportunities for transmission of ASF from WB to DP, and a foundation to conduct a risk assessment of the risks in the Swedish context. While a formal risk assessment was not conducted, the risk characterization allowed us to provide a picture of the existing relevant factors, and the knowledge gaps that need to be addressed.

The most susceptible population to exposure to ASFV if it was circulating in WB seems to be the organic outdoor DP populations. These are relatively small and disperse in Sweden, besides having a transmission temporal window of four months. Farm biosecurity plays a decisive role in most transmission pathways, and due to the fact of that Sweden pig production sector relies on large commercial farms and has a recognized high level of biosecurity (Backhans et al., 2015; EUROSTAT, 2014; Postma et al., 2016), the transmission probability seems reduced. Overall, the opportunities for ASF transmission between the WB and DP population seem to be sporadic, which is in agreement with what has been observed in the Baltic countries (EFSA, 2017). In these countries, backyard and small scale pig farms, usually linked with lower biosecurity standards, have a higher share in total pig population (EUROSTAT, 2014). However epidemics, which are developing mainly in the WB population, have not been observed to often spill into the DP population (EFSA, 2017).

This work has provided a framework to characterize the risk of transmission of ASF from WB to DP in general, complemented by data from Sweden in particular. Knowledge gaps have been identified and highlighted, while data available has shown that there is limited opportunity for effective contact between DP and WB in Sweden.

6. CONCLUSION

The WB can theoretically transmit ASF to susceptible DP trough five distinct pathways: direct contact, indirect contact, infected swill, environmental contamination/local spread and vector borne transmission.

In Sweden, for the direct contact pathway the risk of transmission is related with the outdoor production system, but the potential role of “cold stalls” cannot be ruled out and should be investigated. The risks of transmission by indirect contact are the biosecurity level of the DP farm, the different profiles of human activity in the farm and the high-risk human activities for ASFV contamination. The probability of viral transmission by different profiles of human entering the farm should be considered in a risk assessment context. In the infected swill pathway the key risk factor is the compliance of the pig farmers towards the swill feed ban. The destination of hunted WB could be an important information and therefore it should be assessed in future works together with the level of compliance of swill feed ban on Swedish pig farms. In the environmental contamination/local spread transmission context the risk factors are distance, farm biosecurity and farm type. The potential transmission role of straw should be also subject to future investigations, due to its broad use in Swedish pig farms. The vector borne transmission mediated by the *Ornithodoros* soft tick is thought to not play any role in transmission of ASF at the interface WB-DP in Sweden.

Overall our findings strongly suggest that the potential transmission of ASFV from WB to DP in Sweden is low, and it would occur as a sporadic event, although further research studies should be addressed to this subject.

7. BIBLIOGRAPHY

Achenbach, J. E., Gallardo, C., Nieto-Pelegrín, E., Rivera-Arroyo, B., Degefa-Negi, T., Arias, M., Jenberie, S., Mulisa, D., Gizaw, D., Gelaye, E., Chibassa, T.R., Belaye, A., Loitsch, A., Forsa, M., Yami, M., Diallo, M., Soler, A., Lamien, C.E., Sánchez-Vizcaíno, J. M. (2016). Identification of a New Genotype of African Swine Fever Virus in Domestic Pigs from Ethiopia. *Transboundary and Emerging Diseases*. https://doi.org/10.1111/tbed.12511

Arias, M.L., Escribano, J.M., Rueda, A., Sánchez-Vizcaíno, J.M. (1986). La peste porcina Africana. *Medicine Veterinaire*, 3, 333e350

Backhans, A., Sjölund, M., Lindberg, A., & Emanuelson, U. (2015). Biosecurity level and health management practices in 60 Swedish farrow-to-finish herds. *Acta Veterinaria Scandinavica*, *57*(1), 14. https://doi.org/10.1186/s13028-015-0103-5

Baldacchino, F., Muenworn, V., Desquesnes, M., Desoli, F., Charoenviriyaphap, T., & Duvallet, G. (2013). Transmission of pathogens by *Stomoxys* flies (Diptera, Muscidae): a review. *Parasite*, *20*, 26. https://doi.org/10.1051/parasite/2013026

Bastos, A. D., Penrith, M.-L., Cruciere, C., Edrich, J. L., Hutchings, G., Roger, F., Couacy-Hymann, E., Thomson, G. R. (2003). Genotyping field strains of African swine fever virus by partial p72 gene characterisation. *Archives of Virology*, *148*(4), 693–706.

Bellini, S., Rutili, D., & Guberti, V. (2016). Preventive measures aimed at minimizing the risk of African swine fever virus spread in pig farming systems. *Acta Veterinaria Scandinavica*, *58*(1). https://doi.org/10.1186/s13028-016-0264-x

Beltrán-Alcrudo, D., Guberti, V., de Simone, L., De Castro, J., Rozstalnyy, A., Dietze, K., Wainwright, S., Slingenbergh, J. (2009). African swine fever spread in the Russian Federation and the risk for the region. *EMPRES Watch*. Retrieved from ftp://193.43.36.93/docrep/fao/012/ak718e/ak718e00.pdf

Blome, S., Gabriel, C., & Beer, M. (2013). Pathogenesis of African swine fever in domestic pigs and European wild boar. *Virus Research*, *173*(1), 122–130. https://doi.org/10.1016/j.virusres.2012.10.026

Blome, S., Gabriel, C., Dietze, K., Breithaupt, A., & Beer, M. (2012). High Virulence of African Swine Fever Virus Caucasus Isolate in European Wild Boars of All Ages. *Emerging Infectious Diseases*, *18*(4), 708–708. https://doi.org/10.3201/eid1804.111813

Boinas, F., Ribeiro, R., Madeira, S., Palma, M., de Carvalho, I. L., Núncio, S., & Wilson, A. J. (2014). The medical and veterinary role of Ornithodoros erraticus complex ticks (Acari: Ixodida) on the Iberian Peninsula. *Journal of Vector Ecology*, *39*(2), 238–248. https://doi.org/10.1111/jvec.12098

Boinas, F. S., Wilson, A. J., Hutchings, G. H., Martins, C., & Dixon, L. J. (2011). The Persistence of African Swine Fever Virus in Field-Infected Ornithodoros erraticus during the ASF Endemic Period in Portugal. *PLoS ONE*, *6*(5), e20383. https://doi.org/10.1371/journal.pone.0020383

Boklund, A., Halasa, T., Christiansen, L. E., & Enøe, C. (2013). Comparing control strategies against foot-and-mouth disease: Will vaccination be cost-effective in Denmark? *Preventive Veterinary Medicine*, *111*(3–4), 206–219. https://doi.org/10.1016/j.prevetmed.2013.05.008

Bosch, J., Rodríguez, A., Iglesias, I., Muñoz, M. J., Jurado, C., Sánchez-Vizcaíno, J. M., & de la Torre, A. (2016). Update on the Risk of Introduction of African Swine Fever by Wild Boar into Disease-Free European Union Countries. *Transboundary and Emerging Diseases*. https://doi.org/10.1111/tbed.12527

Boshoff, C. I., Bastos, A. D. S., Gerber, L. J., & Vosloo, W. (2007). Genetic characterisation of African swine fever viruses from outbreaks in southern Africa (1973–1999). *Veterinary Microbiology*, *121*(1–2), 45–55. https://doi.org/10.1016/j.vetmic.2006.11.007

Costard, S., Jones, B. A., Martínez-López, B., Mur, L., de la Torre, A., Martínez, M., Sánchez-Vizcaíno, F., Sánchez-Vizcaíno, J.M., Wieland, B., Gubbins, S. (2013). Introduction of African Swine Fever into the European Union through Illegal Importation of Pork and Pork Products. *PLoS ONE*, *8*(4), e61104. https://doi.org/10.1371/journal.pone.0061104

Costard, S., Mur, L., Lubroth, J., Sanchez-Vizcaino, J. M., & Pfeiffer, D. U. (2013). Epidemiology of African swine fever virus. *Virus Research*, *173*(1), 191–197. https://doi.org/10.1016/j.virusres.2012.10.030

Costard, S., Wieland, B., de Glanville, W., Jori, F., Rowlands, R., Vosloo, W., Roger, F., Pfeiffer, D.U., Dixon, L. K. (2009). African swine fever: how can global spread be prevented? *Philosophical Transactions of the Royal Society B: Biological Sciences*, *364*(1530), 2683–2696. https://doi.org/10.1098/rstb.2009.0098

Davies, K., Goatley, L. C., Guinat, C., Netherton, C. L., Gubbins, S., Dixon, L. K., & Reis, A. L. (2015). Survival of African Swine Fever Virus in Excretions from Pigs Experimentally Infected with the Georgia 2007/1 Isolate. *Transboundary and Emerging Diseases*. https://doi.org/10.1111/tbed.12381

de Carvalho Ferreira, H. C., Backer, J. A., Weesendorp, E., Klinkenberg, D., Stegeman, J. A., & Loeffen, W. L. A. (2013a). Transmission rate of African swine fever virus under experimental conditions. *Veterinary Microbiology*, *165*(3–4), 296–304. https://doi.org/10.1016/j.vetmic.2013.03.026

de Carvalho Ferreira, H. C., Weesendorp, E., Quak, S., Stegeman, J. A., & Loeffen, W. L. A. (2013b). Quantification of airborne African swine fever virus after experimental infection. *Veterinary Microbiology*, *165*(3–4), 243–251. https://doi.org/10.1016/j.vetmic.2013.03.007

de Carvalho Ferreira, H. C., Tudela Zúquete, S., Wijnveld, M., Weesendorp, E., Jongejan, F., Stegeman, A., & Loeffen, W. L. A. (2014). No evidence of African swine fever virus replication in hard ticks. *Ticks and Tick-Borne Diseases*, *5*(5), 582–589. https://doi.org/10.1016/j.ttbdis.2013.12.012

DG SANCO (2013). Guidelines on surveillance and control of African swine fever in feral pigs and preventive measures for pig holdings No. 7138/2013. Retrieved July 15, 2017, from https://ec.europa.eu/food/sites/food/files/animals/docs/ad_control-measures_asf_wrk-doc-sanco-2013-7138.pdf

Dee, S., Otake, S., Oliveira, S., & Deen, J. (2009). Evidence of long distance airborne transport of porcine reproductive and respiratory syndrome virus and *Mycoplasma hyopneumoniae*. *Veterinary Research*, *40*(4), 39. https://doi.org/10.1051/vetres/2009022

Diaz, A. V., Netherton, C. L., Dixon, L. K., & Wilson, A. J. (2012). African Swine Fever Virus Strain Georgia 2007/1 in Ornithodoros erraticus Ticks. *Emerging Infectious Diseases*, *18*(6), 1026–1028. https://doi.org/10.3201/eid1806.111728

Dixon, L. K., Chapman, D. A. G., Netherton, C. L., & Upton, C. (2013). African swine fever virus replication and genomics. *African Swine Fever Virus*, *173*(1), 3–14. https://doi.org/10.1016/j.virusres.2012.10.020

Dohoo, I., Martin, W, & Stryhn, H., 2009. (2009). *Veterinary Epidemiologic Research* (2nd Edition). Charlotte Town, Canada.

EFSA (2009). Scientific review on African swine fever. *EFSA Supporting Publications*, *6*(8). Retrieved from http://onlinelibrary.wiley.com/doi/10.2903/sp.efsa.2009.EN-5/abstract

EFSA Panel on Animal Health and Welfare (AHAW). (2010a). Scientific Opinion on African Swine Fever: African Swine Fever. *EFSA Journal*, *8*(3), 1556. https://doi.org/10.2903/j.efsa.2010.1556

EFSA Panel on Animal Health and Welfare (AHAW). (2010b). Scientific Opinion on the Role of Tick Vectors in the Epidemiology of Crimean-Congo Hemorrhagic Fever and African Swine Fever in Eurasia: Ticks as vectors of CCHF and ASF. *EFSA Journal*, *8*(8), 1703. https://doi.org/10.2903/j.efsa.2010.1703

EFSA Panel on Animal Health and Welfare (AHAW). (2014). Scientific Opinion on African swine fever: African swine fever. *EFSA Journal*, *12*(4), 3628. https://doi.org/10.2903/j.efsa.2014.3628

EFSA Panel on Animal Health and Welfare (AHAW). (2015). African swine fever: African swine fever. *EFSA Journal*, *13*(7), 4163. https://doi.org/10.2903/j.efsa.2015.4163

EFSA, Cortiñas Abrahantes, J., Gogin, A., Richardson, J., & Gervelmeyer, A. (2017). Epidemiological analyses on African swine fever in the Baltic countries and Poland. *EFSA Journal*, *15*(3), e04732. https://doi.org/10.2903/j.efsa.2017.4732

EU (2009) Regulation (EC) No 1069/2009 of the European Parliament and of the Council of 21 October 2009 laying down health rules as regards animal by-products and derived products not intended for human consumption and repealing Regulation (EC) No 1774/2002 (Animal by-products Regulation). Retrieved July 15, 2017, from http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32009R1069&from=EN

EU (2012) Council Directive 2002/60/EC of 27 June 2002 laying down specific provisions for the control of African swine fever and amending Directive 92/119/EEC as regards Teschen disease and African swine fever. Retrieved July 15, 2017, from http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:02002L0060-20080903&from=EN

EU (2016). Facts and figures on organic agriculture in the European Union. Retrieved July 20, 2016, from http://ec.europa.eu/agriculture/rica/pdf/Organic_2016_web_new.pdf

EURL-ASF (2013a). Standard operation procedure for African swine fever virus (ASFV) Isolation on porcine leucocytes and Hemadsportion test. Centro de Investigación en Sanidad Animal, Madrid, Spain. Retrieved July 15, 2017,from http://asf-referencelab.info/asf/images/files/PROTOCOLOS-EN/SOP-ASF-VI-1.pdf

EURL-ASF (2013b). Standard operation procedure for detection of African swine fever virus (ASFV) by conventional polymerase chain reaction (PCR). Centro de Investigación en Sanidad Animal, Madrid, Spain. Retrieved July 15, 2017,from http://asf-referencelab.info/asf/images/files/PROTOCOLOS-EN/SOP-ASF-PCR-1.pdf

EURL-ASF (2013c). Standard operation procedure for detection of African swine fever virus (ASFV) by real-time polymerase chain reaction (PCR). Centro de Investigación en Sanidad Animal, Madrid, Spain. Retrieved July 15, 2017,from http://asf-referencelab.info/asf/images/files/PROTOCOLOS-EN/SOP-ASF-PCR-2.pdf

EURL-ASF (2013d). Standard operation procedure for detection of antibodies against African swine fever by immunoblotting. Centro de Investigación en Sanidad Animal, Madrid, Spain. Retrieved July 15, 2017,from http://asf-referencelab.info/asf/images/files/PROTOCOLOS-EN/SOP-ASF-PCR-2.pdf

EURL-ASF (2013e). Standard operation procedure for detection of antibodies against African swine fever by Indirect ELISA. Centro de Investigación en Sanidad Animal, Madrid, Spain. Retrieved July 15, 2017,from http://asf-referencelab.info/asf/images/files/PROTOCOLOS-EN/SOP-ASF-PCR-2.pdf

EURL-ASF (2016). ASF Diagnostic procedures. Retrieved July 14, 2017, from http://asf-referencelab.info/asf/en/procedures-diagnosis/diagnostic-procedures

EUROSTAT (2014). Pig farming sector - statistical portrait 2014 - Statistics Explained. Retrieved July 22, 2017, from http://ec.europa.eu/eurostat/statistics-explained/index.php/Pig_farming_sector_-_statistical_portrait_2014

FAO EMPRES-I (2017). EMPRES-i - Global Animal Disease Information System. Retrieved July 15, 2017, from http://empres-i.fao.org/eipws3g/

Farez, S., & Morley, R. S. (1997). Potential animal health hazards of pork and pork products. *Revue Scientifique et Technique-Office International Des Épizooties*, *16*, 65–78.

Federation of Swedish Farmers (2015). Swedish Pig Production - A few facts on pig production in Sweden. Federation of Swedish Farmers. Retrieved from https://www.lrf.se/globalassets/dokument/om-lrf/branscher/lrf-kott/grisnaringen/swedish_pig_production_2015.pdf

Früh, B. (2011). *Organic pig production in Europe: health management in common organic pig farming*. Frick: Research Institute of Organic Agriculture (FiBL).

Früh, B., Bochicchio, D., Edwards, S., Hegelund, L., Leeb, C., Sundrum, A., Werne, S., Wiberg, S., Prunier, A. (2014). Description of organic pig production in Europe. *Organic Agriculture*, *4*(2), 83–92. https://doi.org/10.1007/s13165-013-0056-9

Gabriel, C., Blome, S., Malogolovkin, A., Parilov, S., Kolbasov, D., Teifke, J. P., & Beer, M. (2011). Characterization of African Swine Fever Virus Caucasus Isolate in European Wild Boars. *Emerging Infectious Diseases*, *17*(12), 2342–2345. https://doi.org/10.3201/eid1712.110430

Gallardo, C., Blanco, E., Rodriguez, J. M., Carrascosa, A. L., & Sanchez-Vizcaino, J. M. (2006). Antigenic Properties and Diagnostic Potential of African Swine Fever Virus Protein pp62 Expressed in Insect Cells. *Journal of Clinical Microbiology*, *44*(3), 950–956. https://doi.org/10.1128/JCM.44.3.950-956.2006

Gallardo, C., Fernández-Pinero, J., Pelayo, V., Gazaev, I., Markowska-Daniel, I., Pridotkas, G., Nieto, R., Fernández-Pacheco, P., Bokhan, S., Nevolko, O., Drozhzhe, Z., Pérez, C., Soler, A., Kolvasov, D., Arias, M. (2014). Genetic Variation among African Swine Fever Genotype II Viruses, Eastern and Central Europe. *Emerging Infectious Diseases*, *20*(9), 1544–1547. https://doi.org/10.3201/eid2009.140554

Gallardo, C., Nieto, R., Soler, A., Pelayo, V., Fernández-Pinero, J., Markowska-Daniel, I., Pridotkas, G., Nurmoja, I., Granta, R., Simón, A., Pérez, C., Martín, E., Fernández-Pacheco, P., Arias, M. (2015a). Assessment of African Swine Fever Diagnostic Techniques as a Response to the Epidemic Outbreaks in Eastern European Union Countries: How To Improve Surveillance and Control Programs. *Journal of Clinical Microbiology*, *53*(8), 2555–2565. https://doi.org/10.1128/JCM.00857-15

Gallardo, C., Reoyo, A. de la T., Fernández-Pinero, J., Iglesias, I., Muñoz, M. J., Arias, M. L. (2015b). African swine fever: a global view of the current challenge. *Porcine Health Management*, *1*(1). https://doi.org/10.1186/s40813-015-0013-y

Gallardo, C., Soler, A., Nieto, R., Cano, C., Pelayo, V., Sánchez, M. A., Fernandez-Pinero, J., Briones, V., Arias, M. (2015c). Experimental Infection of Domestic Pigs with African Swine Fever Virus Lithuania 2014 Genotype II Field Isolate. *Transboundary and Emerging Diseases*, *64*(1), 300–304. https://doi.org/10.1111/tbed.12346

Garner, M. G., & Cannon, R. M. (1995). Potential for wind-borne spread of foot-and-mouth disease virus in Australia. *A Report Prepared for the Australian Meat Research Corporation, Bureau of Resource Sciences, Canberra*. Retrieved from http://www.agriculture.gov.au/SiteCollectionDocuments/animal-plant/pests-diseases/fmd/fmdwind.pdf

Gavier-Widén, D., Gortázar, C., Ståhl, K., Neimanis, A. S., Rossi, S., H\a ard av Segerstad, C., & Kuiken, T. (2015). African swine fever in wild boar in Europe: a notable challenge. *Vet Rec*, *176*, 199–200.

Gogin, A., Gerasimov, V., Malogolovkin, A., & Kolbasov, D. (2013). African swine fever in the North Caucasus region and the Russian Federation in years 2007–2012. *Virus Research*, *173*(1), 198–203. https://doi.org/10.1016/j.virusres.2012.12.007

Government Offices of Sweden (2017). Retrieved July 15, 2017, from: http://www.government.se/government-agencies/county-administrative-boards-lansstyrelserna/

Gren, I.-M., Häggmark-Svensson, T., Andersson, H., Jansson, G., & Jägerbrand, A. (2016). Using traffic data to estimate wildlife populations. *Journal of Bioeconomics*, *18*(1), 17–31. https://doi.org/10.1007/s10818-015-9209-0

Guberti, V. (2016). *Biosecurity during hunting, carcass disposal and population management*. Presented at the Sixth meeting of the Standard Group of Experts on ASF in the Baltic and Eastern Europe Region, Vilnius, Lithuania. Retrieved from http://web.oie.int/rr-europe/eng/Regprog/docs/docs/SG6/SGE%20ASF6%20-%20Hunting%20BioS%20(V.%20Guberti).pdf

Guinat, C., Reis, A. L., Netherton, C. L., Goatley, L., Pfeiffer, D. U., & Dixon, L. (2014). Dynamics of African swine fever virus shedding and excretion in domestic pigs infected by intramuscular inoculation and contact transmission. *Veterinary Research*, *45*(1), 1.

Guinat, C., Gubbins, S., Vergne, T., Gonzales, J. L., Dixon, L., & Pfeiffer, D. U. (2016a). Experimental pig-to-pig transmission dynamics for African swine fever virus, Georgia 2007/1 strain. *Epidemiology and Infection*, *144*(1), 25–34. https://doi.org/10.1017/S0950268815000862

Guinat, C., Gogin, A., Blome, S., Keil, G., Pollin, R., Pfeiffer, D., & Dixon, L. (2016b). Transmission routes of African swine fever virus to domestic pigs: current knowledge and future research directions. *Veterinary Record*, *178*(11), 262–267. https://doi.org/10.1136/vr.103593

Guinat, C., Vergne, T., Jurado-Diaz, C., Sánchez-Vizcaíno, J. M., Dixon, L., & Pfeiffer, D. U. (2017). Effectiveness and practicality of control strategies for African swine fever: what do we really know? *Veterinary Record*, *180*(4), 97–97. https://doi.org/10.1136/vr.103992

Halasa, T., Boklund, A., Stockmarr, A., Enøe, C., & Christiansen, L. E. (2014). A Comparison between Two Simulation Models for Spread of Foot-and-Mouth Disease. *PLoS ONE*, *9*(3), e92521. https://doi.org/10.1371/journal.pone.0092521

Haresnape, J. M., & Mamu, F. D. (1986). The distribution of ticks of the Ornithodoros moubata complex (Ixodoidea: Argasidae) in Malawi, and its relation to African swine fever epizootiology. *Journal of Hygiene*, *96*(3), 535–544. https://doi.org/10.1017/S0022172400066341

Hoinville, L. J., Alban, L., Drewe, J. A., Gibbens, J. C., Gustafson, L., Häsler, B., Saegerman, C., Salman, C., Stärk, K. D. C. (2013). Proposed terms and concepts for describing and evaluating animal-health surveillance systems. *Preventive Veterinary Medicine*, *112*(1–2), 1–12. https://doi.org/10.1016/j.prevetmed.2013.06.006

International Committee on Taxonomy of Viruses, & King, A. (Eds.) (2012). *Virus taxonomy: classification and nomenclature of viruses: ninth report of the International Committee on Taxonomy of Viruses*. London ; Waltham, MA: Academic Press.

Jord på Trynet. (2017). Retrieved July 15, 2017, from: http://www.jordpatrynet.se

Jori, F., & Bastos, A. (2009). Role of Wild Suids in the Epidemiology of African Swine Fever. *EcoHealth*, *6*(2), 296–310. https://doi.org/10.1007/s10393-009-0248-7

Laddomada, A., Patta, C., Oggiano, A., Caccia, A., Ruiu, A., Cossu, P., & Firinu, A. (1994). Epidemiology of classical swine fever in Sardinia: a serological survey of wild boar and comparison with African swine fever. *Veterinary Record*, *134*(8), 183–187. https://doi.org/10.1136/vr.134.8.183

Khomenko, S., Beltrán-Alcrudo, D., Rozstalnyy, A., Gogin, A., Kolbasov, D., Pinto, J., Lubroth, J., Martin, V. (2013). African swine fever in the Russian Federation: risk factors. *Emprest Watch Fao*, *28*. Retrieved from http://www.fao.org/docs/eims/upload/310776/aq240e.pdf

Lemel, J. (1999) Populationstillväxt, dynamik och spridning hos vildsvinet, *Sus scrofa*, i mellersta Sverige - Report, Swedish Environmental Protection Agency in Magnusson, M. (2010). *Population and management models for the Swedish wild boar (Sus scrofa).* Master ́s thesis, Department of Ecology. Uppsala/Grimsö: Swedish University of Agricultural Sciences, The Faculty of Natural Resources and Agricultural. Retrieved from http://stud.epsilon.slu.se/2041/5/magnusson_m_101123.pdf

Lyra, T.M.P. (2006). The eradication of African swine fever in Brazil, 1978–1984. *Rev Sci Tech Off Int Epizoot* 25: 93–103 in Costard, S., Mur, L., Lubroth, J., Sanchez-Vizcaino, J. M., & Pfeiffer, D. U. (2013). Epidemiology of African swine fever virus. *Virus Research*, *173*(1), 191–197. https://doi.org/10.1016/j.virusres.2012.10.030

Lubisi, B. A., Bastos, A. D. S., Dwarka, R. M., & Vosloo, W. (2005). Molecular epidemiology of African swine fever in East Africa. *Archives of Virology*, *150*(12), 2439–2452. https://doi.org/10.1007/s00705-005-0602-1

Magnusson, M. (2010). *Population and management models for the Swedish wild boar (Sus scrofa).* Master ́s thesis, Department of Ecology. Uppsala/Grimsö: Swedish University of Agricultural Sciences, The Faculty of Natural Resources and Agricultural. Retrieved from http://stud.epsilon.slu.se/2041/5/magnusson_m_101123.pdf

Malmquist, W. A., & Hay, D. (1960). Hemadsorption and cytopathic effect produced by African swine fever virus in swine bone marrow and buffy coat cultures. *American Journal of Veterinary Research*, *21*(104–108).

Manso Ribero, J., Nunes Petisca, J. L., Lopes Frazão, F., & Sobral, M. (1963). Vaccination contre la peste porcine. *Bull. Off. Int. Epizoo.*, *60*, 921–937.

Mebus, C., Arias, M., Pineda, J. M., Tapiador, J., House, C., & Sánchez-Vizcaíno, J. M. (1997). Survival of several porcine viruses in different Spanish dry-cured meat products. *Food Chemistry*, *59*(4), 555–559. https://doi.org/10.1016/S0308-8146(97)00006-X

Moulton J, Coggins L (1968) Comparison of lesions in acute and chronic African swine fever. *Cornell Veterinarian*, 58, 364-387

Mur, L., Boadella, M., Martínez-López, B., Gallardo, C., Gortazar, C., & Sánchez-Vizcaíno, J. M. (2012). Monitoring of African Swine Fever in the Wild Boar Population of the Most Recent Endemic Area of Spain. *Transboundary and Emerging Diseases*, *59*(6), 526–531. https://doi.org/10.1111/j.1865-1682.2012.01308.x

SVA. (2011). Surveillance of infectious diseases in animals and humans in Sweden 2011. Retrieved from http://www.sva.se/globalassets/redesign2011/pdf/om_sva/publikationer/surveillance2011.pdf

Nöremark, M., Lindberg, A., Vågsholm, I., & Sternberg Lewerin, S. (2009). Disease awareness, information retrieval and change in biosecurity routines among pig farmers in association with the first PRRS outbreak in Sweden. *Preventive Veterinary Medicine*, *90*(1–2), 1–9. https://doi.org/10.1016/j.prevetmed.2009.03.008

Nöremark, M., Frössling, J., & Lewerin, S. S. (2010). Application of Routines that Contribute to On-farm Biosecurity as Reported by Swedish Livestock Farmers: On-farm Biosecurity as Reported by Swedish Livestock Farmers. *Transboundary and Emerging Diseases*, no-no. https://doi.org/10.1111/j.1865-1682.2010.01140.x

Nöremark, M., Frössling, J., & Lewerin, S. S. (2013). A survey of visitors on Swedish livestock farms with reference to the spread of animal diseases. *BMC Veterinary Research*, *9*(1), 184.

Nöremark, M., & Sternberg-Lewerin, S. (2014). On-farm biosecurity as perceived by professionals visiting Swedish farms. *Acta Veterinaria Scandinavica*, *56*(1), 28.

Nurmoja, I., Petrov, A., Breidenstein, C., Zani, L., Forth, J. H., Beer, M., … Blome, S. (2017). Biological characterization of African swine fever virus genotype II strains from north-eastern Estonia in European wild boar. *Transboundary and Emerging Diseases*. https://doi.org/10.1111/tbed.12614

Oganesyan, A. S., Petrova, O. N., Korennoy, F. I., Bardina, N. S., Gogin, A. E., & Dudnikov, S. A. (2013). African swine fever in the Russian Federation: Spatio-temporal analysis and epidemiological overview. *Virus Research*, *173*(1), 204–211. https://doi.org/10.1016/j.virusres.2012.12.009

OIE. (2012). Chapter 2.8.1 - African Swine Fever. In *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2017* (pp. 1067–1081). Paris, France: World Organisation for Animal Health (OIE). Retrieved from http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.08.01_ASF.pdf

OIE. (2013). African Swine Fever - OIE Technical Disease Cards. Retrieved from https://www.oie.int/fileadmin/Home/eng/Animal_Health_in_the_World/docs/pdf/Disease_cards/AFRICAN_SWINE_FEVER.pdf

OIE. (2016). Chapter 15.1 - African swine fever. In *OIE Terrestrial Animal Health Code* (OIE, Vol. 2, p. 6). Paris, France. Retrieved from http://www.oie.int/index.php?id=169&L=0&htmfile=chapitre_asf.htm

Oļševskis, E., Guberti, V., Seržants, M., Westergaard, J., Gallardo, C., Rodze, I., & Depner, K. (2016). African swine fever virus introduction into the EU in 2014: Experience of Latvia. *Research in Veterinary Science*, *105*, 28–30. https://doi.org/10.1016/j.rvsc.2016.01.006

Oravainen, J., Sahlström, L., & Lyytikäinen, T. (2011). Afrikkalaisen sikaruton mahdollisia maahantuloreittejä. Retrieved from https://helda.helsinki.fi/handle/10138/27421

Oura, C. A. L., Edwards, L., & Batten, C. A. (2013). Virological diagnosis of African swine fever—Comparative study of available tests. *Virus Research*, *173*(1), 150–158. https://doi.org/10.1016/j.virusres.2012.10.022

Oura, C. A., Powell, P. P., & Parkhouse, R. M. (1998). African swine fever: a disease characterized by apoptosis. *Journal of General Virology*, *79*(6), 1427–1438.

Pastor, M. J., Laviada, M. D., Sanchez-Vizcaino, J. M., & Escribano, J. M. (1989). Detection of African swine fever virus antibodies by immunoblotting assay. *Canadian Journal of Veterinary Research*, *53*(1), 105.

Pejsak, Z., Truszczyński, M., Niemczuk, K., Kozak, E., & Markowska-Daniel, I. (2014). Epidemiology of African Swine Fever in Poland since the detection of the first case. *Polish Journal of Veterinary Sciences*, *17*(4). https://doi.org/10.2478/pjvs-2014-0097

Penrith, M.-L., & Vosloo, W. (2009). Review of African swine fever: transmission, spread and control : review article. *Journal of the South African Veterinary Association*, *80*(2). https://doi.org/10.4102/jsava.v80i2.172

Pietschmann, J., Guinat, C., Beer, M., Pronin, V., Tauscher, K., Petrov, A., … Blome, S. (2015). Course and transmission characteristics of oral low-dose infection of domestic pigs and European wild boar with a Caucasian African swine fever virus isolate. *Archives of Virology*, *160*(7), 1657–1667. https://doi.org/10.1007/s00705-015-2430-2

Pietschmann, J., Mur, L., Blome, S., Beer, M., Pérez-Sánchez, R., Oleaga, A., & Sánchez-Vizcaíno, J. M. (2016). African swine fever virus transmission cycles in Central Europe: Evaluation of wild boar-soft tick contacts through detection of antibodies against Ornithodoros erraticus saliva antigen. *BMC Veterinary Research*, *12*(1). https://doi.org/10.1186/s12917-015-0629-9

Plowright, W. (1986). African swine fever: a retrospective view. *Scientific and Technical Review of the Office International Des Epizooties*, *5*(2), 455–468.

Postma, M., Backhans, A., Collineau, L., Loesken, S., Sjölund, M., Belloc, C., Emanuelson, U., Beilage, E., Stärk, K., Dewulf, J. (2016). The biosecurity status and its associations with production and management characteristics in farrow-to-finish pig herds. *Animal*, *10*(3), 478–489. https://doi.org/10.1017/S1751731115002487

Probst, C., Globig, A., Knoll, B., Conraths, F. J., & Depner, K. (2017). Behaviour of free ranging wild boar towards their dead fellows: potential implications for the transmission of African swine fever. *Royal Society Open Science*, *4*(5), 170054. https://doi.org/10.1098/rsos.170054

Rahimi, P., Sohrabi, A., Ashrafihelan, J., Edalat, R., Alamdari, M., Masoudi, M., Mostofi, S., Azadmanesh, K. (2010). Emergence of African Swine Fever Virus, Northwestern Iran. *Emerging Infectious Diseases*, *16*(12), 1946–1948. https://doi.org/10.3201/eid1612.100378

Rowlands, R. J., Michaud, V., Heath, L., Hutchings, G., Oura, C., Vosloo, W., Dwarka, R., Onashvili, T., Albina, E., Dixon, L. K. (2008). African Swine Fever Virus Isolate, Georgia, 2007. *Emerging Infectious Diseases*, *14*(12), 1870–1874. https://doi.org/10.3201/eid1412.080591

Salajpal, K., Karolyi, D., & Luković, Z. (2013). Sanitary aspects of outdoor farming systems. *Acta Agriculturae Slovenica*, *4*, 109–117.

Sánchez-Botija, C. (1982). African swine fever. New developments. *Revue Scientifique et Technique*, 4, 1065-1094

Sánchez-Vizcaino JM (2006) African swine fever. Diseases of Swine. Blackwell Publishing, pp 291–298 in OIE. (2012). Chapter 2.8.1 - African Swine Fever. In *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2017* (pp. 1067–1081). Paris, France: World Organisation for Animal Health (OIE). Retrieved from http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.08.01_ASF.pdf

Sánchez-Vizcaíno, J. M., Mur, L., & Martínez-López, B. (2012). African Swine Fever: An Epidemiological Update: African Swine Fever: An Epidemiological Update. *Transboundary and Emerging Diseases*, *59*, 27–35. https://doi.org/10.1111/j.1865-1682.2011.01293.x

Sánchez-Vizcaíno, J. M., & Mur, L. (2013). African Swine Fever Diagnosis Update, *135*, 159–165. https://doi.org/10.1159/000189240 in Gallardo, C., Nieto, R., Soler, A., Pelayo, V., Fernández-Pinero, J., Markowska-Daniel, I., Pridotkas, G., Nurmoja, I., Granta, R., Simón, A., Pérez, C., Martín, E., Fernández-Pacheco, P., Arias, M. (2015a). Assessment of African Swine Fever Diagnostic Techniques as a Response to the Epidemic Outbreaks in Eastern European Union Countries: How To Improve Surveillance and Control Programs. *Journal of Clinical Microbiology*, *53*(8), 2555–2565. https://doi.org/10.1128/JCM.00857-15

Sánchez-Vizcaíno, J. M., Mur, L., & Martínez-López, B. (2013). African swine fever (ASF): Five years around Europe. *Veterinary Microbiology*, *165*(1–2), 45–50. https://doi.org/10.1016/j.vetmic.2012.11.030

Sánchez-Vizcaíno, J. M., Mur, L., Gomez-Villamandos, J. C., & Carrasco, L. (2015a). An Update on the Epidemiology and Pathology of African Swine Fever. *Journal of Comparative Pathology*, *152*(1), 9–21. https://doi.org/10.1016/j.jcpa.2014.09.003

Sánchez-Vizcaíno, J. M., Mur, L., Bastos, A. D., & Penrith, M. L. (2015b). New insights into the role of ticks in African swine fever epidemiology. *Rev Sci Tech*, *34*, 503–511.

Śmietanka, K., Woźniakowski, G., Kozak, E., Niemczuk, K., Frączyk, M., Bocian, Ł., … Pejsak, Z. (2016). African Swine Fever Epidemic, Poland, 2014–2015. *Emerging Infectious Diseases*, *22*(7), 1201–1207. https://doi.org/10.3201/eid2207.151708

Steiger, Y., Ackermann, M., Mettraux, C., & Kihm, U. (1992). Rapid and biologically safe diagnosis of African swine fever virus infection by using polymerase chain reaction. *Journal of Clinical Microbiology*, *30*(1), 1–8.

SVA (2011). Surveillance of infectious diseases in animals and humans in Sweden 2011. Retrieved July 15, 2017, from http://www.sva.se/globalassets/redesign2011/pdf/om_sva/publikationer/surveillance2011.pdf

SVA (2012). Surveillance of infecious diseases in animals and humans in Sweden 2012. Retrieved July 15, 2017, from http://www.sva.se/globalassets/redesign2011/pdf/om_sva/publikationer/surveillance2012.pdf

SVA (2013). Surveillance of infecious diseases in animals and humans in Sweden 2013. Retrieved July 15, 2017, from http://www.sva.se/globalassets/redesign2011/pdf/om_sva/publikationer/surveillance2013_w.pdf

SVA (2014). Surveillance of infecious diseases in animals and humans in Sweden 2014. *SVA:s Rapportserie 31, National Veterinary Institute (SVA)*. Retrieved July 15, 2017, from http://www.sva.se/globalassets/redesign2011/pdf/om_sva/publikationer/surveillance2014.pdf

SVA (2015). Surveillance of infecious diseases in animals and humans in Sweden 2015. *SVA:s Rapportserie 34*. Retrieved July 15, 2017, from http://www.sva.se/globalassets/redesign2011/pdf/om_sva/publikationer/surveillance-2015-w.pdf

Sverige Länsgränser (2017) Retrieved 20 July, 2017, from http://www.arcgis.com/home/item.html?id=912b806e3b864b5f83596575a2f7cb01

Swedish Association for Hunting and Wildlife Management (Svenska Jägareförbundet). (2016). Retrieved January 1, 2017, from https://jagareforbundet.se/jakten/jakt-olika-viltslag2/hunting-in-sweden/#hunting2.

Swedish Association for Hunting and Wildlife Management (Svenska Jägareförbundet). (2017). Viltdata. Retrieved January 2, 2017, from https://rapport.viltdata.se/statistik/

Swedish Board of Agriculture. (2014). *Ekologisk produktion* (Jordbruksstatistisk Årsbok 2014). Retrieved July 15, 2017, from http://www.jordbruksverket.se/download/18.37e9ac46144f41921cd26f97/1406032002174/Kap+11+Ekologisk+produktion.pdf

Swedish Board of Agriculture. (2016a). *Ekologisk animalieproduktion 2015* (JO - Jordbruk, skogsbruk och fiske.) (p. 17). Swedish Board of Agriculture. Retrieved July 15, 2017, from https://www.jordbruksverket.se/webdav/files/SJV/Amnesomraden/Statistik,%20fakta/Animalieproduktion/JO27SM1601/JO27SM1601.pdf

Swedish Board of Agriculture. (2016b). Företag med husdjur efter län/riket och djurslag. År 2000-2016. Retrieved July 16, 2016, from http://statistik.sjv.se/PXWeb/pxweb/sv/Jordbruksverkets%20statistikdatabas/Jordbruksverkets%20statistikdatabas__Husdjur__Antal%20foretag%20med%20husdjur/JO0103G1.px/?rxid=5adf4929-f548-4f27-9bc9-78e127837625%20?

Swedish Environmental Protection Agency. (2014). *Food Waste quantities in Sweden 2012* (p. 20). Swedish Environmental Protection Agency (Naturvardsverket). Retrieved July 15, 2017, from http://www.naturvardsverket.se/Om-Naturvardsverket/Publikationer/ISBN/8600/978-91-620-8695-4/

Thrusfield M. (2005). *Veterinary Epidemiology* (3rd ed.). Ames, Iowa, USA.

Tulman, E. R., Delhon, G. A., Ku, B. K., & Rock, D. L. (2009). African Swine Fever Virus. In J. L. Van Etten (Ed.), *Lesser Known Large dsDNA Viruses* (pp. 43–87). Berlin, Heidelberg: Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-540-68618-7_2

Vaduva, G. (2015). A study on bloodsucking Tabanidae and Stomoxys calcitrans (Diptera) attacking horses and cows in Northern Scania, Sweden. *Journal of Biology and Life Science*, *7*(1), 19. https://doi.org/10.5296/jbls.v7i1.8611

Vergne, T., Gogin, A., & Pfeiffer, D. U. (2015). Statistical Exploration of Local Transmission Routes for African Swine Fever in Pigs in the Russian Federation, 2007-2014. *Transboundary and Emerging Diseases*, *64*(2), 504–512. https://doi.org/10.1111/tbed.12391

Villeda, C. J., Williams, S. M., Wilkinson, P. J., & Vinuela, E. (1993). Consumption coagulopathy associated with shock in acute African swine fever. *Archives of Virology*, *133*(3), 467–475.

Vose, D. (2008). *Risk Analysis: A Quantitative Guide* (3rd ed.). John Wiley and Sons.

Wallander, C., Frössling, J., Dórea, F. C., Uggla, A., Vågsholm, I., & Lundén, A. (2016). Pasture is a risk factor for Toxoplasma gondii infection in fattening pigs. *Veterinary Parasitology*, *224*, 27–32. https://doi.org/10.1016/j.vetpar.2016.05.005

Wallgren, T., Westin, R., & Gunnarsson, S. (2016). A survey of straw use and tail biting in Swedish pig farms rearing undocked pigs. *Acta Veterinaria Scandinavica*, *58*(1). https://doi.org/10.1186/s13028-016-0266-8

Wiklund, E., & Malmfors, G. (2014). 26. Game meat as a resource in Sweden – with particular focus on moose (*Alces alces*). In P. Paulsen, A. Bauer, & F. J. M. Smulders (Eds.), *Trends in game meat hygiene* (pp. 305–320). The Netherlands: Wageningen Academic Publishers. https://doi.org/10.3920/978-90-8686-238-2_26

Wilkinson, P. J., & Donaldson, A. I. (1977). Transmission studies with African swine fever virus: The early distribution of virus in pigs infected by airborne virus. *Journal of Comparative Pathology*, *87*(3), 497–501.

Williams, N. (2009). African swine fever – outbreak in the Caucasus and why Australia should be aware. *Australian Veterinary Journal*, *87*(11), 18–19.

Woźniakowski, G., Frączyk, M., Niemczuk, K., & Pejsak, Z. (2016). Selected aspects related to epidemiology, pathogenesis, immunity, and control of African swine fever. *Journal of Veterinary Research*, *60*(2). https://doi.org/10.1515/jvetres-2016-0017